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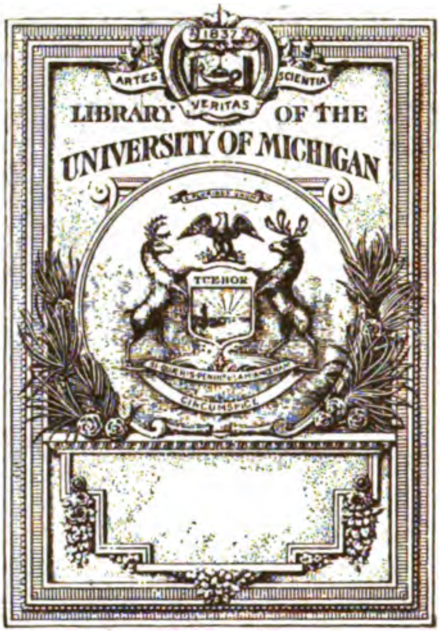

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The following contributions have been published during the years 1914-1915:

Baumgartner, E. A. 1. The development of the hypophysis in Squalus acanthias. *Journal of Morphology,* 26 (1915): 391-446, 43 figures.

2. The development of the gall bladder and bile ducts in Amblystoma. (Abstract.) *Anatomical Record,* 8 (1914).

Hoskins, E. R. 1. On the vascularization of the spinal cord of the pig. *Anatomical Record,* 8 (1914): 371-391, 5 figures.

2. Persistent arteriae brachii superficialis, antibrachii superficialis et mediana. *Anatomical Record,* 8 (1914): 421-422, 1 figure.

3. On the development of the digitiform gland in Squalus acanthias. (Abstract.) *Anatomical Record,* 9 (1915).

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2. Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. (Abstract.) *Anatomical Record,* 9 (1915).

3. Changes in young albino rats held at constant body-weight by underfeeding for various periods. (Abstract.) *Anatomical Record,* 9 (1915).

4. Effects of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. *American Journal of Anatomy,* 18 (1915): 75-116, 2 figures.

5. Changes in the relative weights of the various parts, systems, and organs of young albino rats held at constant body-weight by under-feeding for various periods. *Journal of Experimental Zoology,* 19 (1915): 99-156, 4 figures.

6. Obstacles to research. *Science,* N.S., 42: 819-823, (Dec. 10, 1915).

Johnston, J. B. 1. The nervus terminalis in man and mammals. *Anatomical Record,* 8 (1914): 185-198, 9 figures.

2. A tractus olfacto-tegmentalis in the human fetal brain. *Journal of Comparative Neurology,* 25 (1915): 283-290, 9 figures.

3. The cell masses in the forebrain of the turtle, Cistudo carolina. *Journal of Comparative Neurology,* 25 (1915): 393-468, 60 figures.

Lee, Thomas G. 1. On the implantation and placentation in the sciuroid rodents. (Abstract.) *Anatomical Record,* 9 (1915).

2. On the relationship of the endocardium to entoderm in Citellus. (Abstract.) *Anatomical Record,* 9 (1915).

3. The passage of the ovum through the uterine epithelium in Geomys bursarius, with demonstration of wax reconstructions. (Abstract.) *Anatomical Record,* 8 (1914).

4. An improved electric microscope lamp, with demonstration. (Abstract.) *Anatomical Record,* 8 (1914).

Myers, J. A. 1. Studies on the syrinx of Gallus domesticus. (Abstract.) *Anatomical Record,* 9 (1915).

Scammon, Richard E. 1. The development of the pancreas in selachians. (Abstract.) *Anatomical Record,* 8 (1914).

2. The technique of Weber's method of reconstruction. (Abstract.) *Anatomical Record,* 9 (1915).

3. On Weber's method of reconstruction and its application to curved surfaces. *Anatomical Record,* 9 (1915) : 247-258, 5 figures.

4. The histogenesis of the selachian liver. *American Journal of Anatomy,* 17 (1915) : 245-315, 45 figures, 7 plates.

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THE DEVELOPMENT OF THE HYPOPHYSIS IN SQUALUS ACANTHIAS

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FORTY-THREE FIGURES

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INTRODUCTION

Although considerable work has been done on the hypophysis of elasmobranchs, many points still remain concerning which there has been much discussion and which evidently require further investigation. It was thought that an intensive study of the history of this organ in one form might be of value.

The embryos used are from the collection of Dr. R. E. Scammon and from the large series which form a part of the embryological collection of the Harvard Medical School.[2] For the pup and adult stages special sections and dissections were made. The difficulties in making a dissection of the hypophysis in selachians have been noted by other investigators. In the present work, it was possible only after numerous attempts to obtain a dissection showing the ventral lobes connected with the rest of the hypophysis. Graphic and wax reconstructions of different embryos and parts of the adult Squalus were made.

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[1] This work was begun while at the University of Minnesota and completed at Washington University Medical School.

LITERATURE

1. *Embryology*

The literature on the development of the elosmobranch hypophysis may properly be divided into two categories, one on the embryology and the other on the adult anatomy and histology of this organ. Although the earlier work on the hypophysis in elasmobranchs concerned its adult anatomy, probably investigators have occupied themselves more with its development. Some, of course, have given attention to both the adult anatomy and the development in the same paper or in a series of articles.

Müller ('71) confirmed Rathke's earlier observations that the hypophysis is developed from the mouth. In Acanthias vulgaris and other selachians he found that it was composed of a principal posterior part and a secondary anterior part. He described the position and gave measurements of the size of the hypophysis and of the thickness of different parts of the wall in an Acanthias embryo 30 mm. long. In a 10 cm. embryo of Mustelus he described the tubular masses forming the hypophysis.

Balfour ('74) briefly stated that the hypophysis is an outpouching from the mouth. In 1878 he described the hypophysis as being somewhat constricted from the mouth by the close of

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[2] Through the courtesy of the late Dr. Charles S. Minot I was able to make use of the extensive Harvard Embryological Collection and had the privileges of his laboratory during the summer of 1914. I also wish to thank Dr. J. S. Kingsley and others of the South Harpswell Laboratory for the courtesies of that laboratory for a part of the summer.

'Stage K.' Soon after this its terminal part dilates and the hypophysis is completely constricted from the mouth.

Reichert ('77) in a description of some cleared embryos of Acanthias remarked on the position of the hypophysis and its relation to the notochord. He opposed the theory of the ectodermal origin of the hypophysis in Acanthias.

Rabl-Rückhard ('80) in his work on the relations of the notochord, confirmed Müller's observations concerning the hypophysis. He stated that there is a ventral secondary outpouching from the hypophysis in a 60 mm. Acanthias. In an embryo corresponding to Stage K of Balfour he has perhaps mistaken the connection between the premandibular somites for the hypophysis. This seems probable from the position as well as from the fact that he found no connection between this structure and the mouth at this time.

In 1888 Edinger published a paper on the comparative anatomy of the forebrain. He stated that in Torpedo the hypophysis is at first a simple outpouching of the mouth which later develops secondary outpouchings.

Sedgwick ('92) in his work on Scyllium and Raia made the statement that the first rudiment of the mouth extends into the pituitary body.

Von Kupffer ('94) reviewed the literature on the development of the hypophysis and gave some results of his own work. He thought that the hypophysis is not derived entirely from ectoderm, and his observations on other vertebrates supported his view that the hypophysis is partly of entodermal origin.

Hoffmann ('96) described the early stages in Acanthias development. He stated that the hypophysis develops entirely from ectoderm. He found the first definite outpouching anterior to the buccopharyngeal membrane in 13 to 14 mm. embryos. He also described a yellowish pigment in the buccopharyngeal membrane which could still be seen in the stalk of 25 mm. embryos.

Haller ('96) gave an account of the development of the hypophysis in Mustelus. He described the position of the organ in a 22 mm. embryo and called attention to the low epithelium found at the opening of the hypophysis into the mouth. The connection with the mouth is soon lost and in a 90 mm. stage the whole structure has come into closer relation with the brain floor and vascular sac, and has grown forward. The roof of the hypophysis has become thickened, as have the anterior and posterior ends. From the floor of the caudal end two lateral outpouchings have developed which later form the inferior sacs. In a 20 cm. Mustelus all parts of the hypophysis are quite well developed. A vascular layer separates the roof from the brain floor. The roof of the inferior sacs becomes thinner. Haller also described the interhypophyseal canal joining the inferior sacs to the superior part. He figured glandular outgrowths extending forward from it. From the floor of the anterior sac are several prolongations extending anteriorly while from the roof are many small outpouchings. The extreme anterior end shows many mitotic figures and is broken up into a network by many capillaries. The floor at the cephalic end of the anterior lobe is very thin—this, Haller stated, is where the hypophysis is constricted from the mouth and here the epithelium has remained of a low cuboidal type. The head of the organ is composed of many closely-crowded tubules. These develop first as solid evaginations, then the nuclei separate and rearrange themselves, later a cleft appears in the protoplasm, which cleft ultimately connects with the main lumen.

Chiarugi ('98) briefly considered the hypophysis in a paper on the description of a prehypophyseal body and the hypophyseal area in Torpedo ocellata. He described a connection between the premandibular somites and the hypophysis and figured a median sagittal section of a 15 mm. embryo which showed a constriction of the hypophysis from the mouth.

Nishikawa ('99) noted that the hypophysis is present as a simple outpouching in 32 mm. Chlamydoselachus embryos. He made a series of drawings of transverse sections which show its position at this stage.

Sewertzoff ('99) in studying the development of the selachian skull took up the interrelation of development of the skull and brain. The trabeculae and parachordal plates in Acanthias and Pristiurus are almost at right angles to each other in early stages (figs. 25 and 29). Later a bend in the medulla and a corresponding one in the parachordal plate changes the relation of the two cartilages. Also the forebrain has shifted from a position ventral to the medulla to a more dorsal and rostral one (fig. 1). Sewertzoff showed the change in position of the hypophysis occurring with the change in position of the skull and brain (fig. 23).

Rossi ('02) described the development of two lateral lobes in Torpedo. These, he stated, develop very early from the lateral walls of the evagination forming the hypophysis. He homologized the different portions of the hypophysis as he found them with those described by Haller, but described two special lateral lobes which he stated have no homologous parts in Mustelus according to Haller's description.

Gentes ('06, '07) found a close relationship between the vascular sac and the underlying hypophysis. In several short reports ('08) he described the lateral lobes and the development of the inferior lobes of the hypophysis. These two parts, he stated, form a ventral pituitary body. In a longer paper this author ('08) gave the results of his studies on the development and evolution of the hypophysis in Torpedo. He described two main parts, the superior and inferior sacs. The superior sac is further divided into posterior and anterior parts. The hypophysis begins as an outpouching which in 45 mm. embryos shows a beginning of its division into the two main sacs. From the posterior part of the superior sac many cords grow dorsalward, these later becoming tubular. Gentes homologized the superior lobe with the anterior lobe of mammals. The lateral lobes he believed persist through life.

Ziegler ('08) in a series of figures of an embryo of Chlamydoselachus, corresponding to Balfour's Stage L–M, showed the hypophysis as an upward anterior-extending evagination from the mouth. The connection with the mouth is still a wide canal.

Johnston ('09) described the hypophysis in Acanthias as consisting of a short anterior portion which grows toward the optic chiasma, and a longer posterior lobe directed toward the vascular sac.

Scammon ('11) in the normal plate series has figured the hypophysis in several of the younger stages. He described a distinct outpouching in 7.5 mm. embryos. In 18 mm. embryos a constriction of the anterior part of the hypophysis from the mouth has begun. In 24 mm. embryos shallow furrows separate two lateral portions from a median portion in the posterior part. A slight lateral constriction separates the anterior and posterior lobes.

In 1912 Sterzi described the development of the hypophysis in Acanthias. He noted that the front wall of the outpouching becomes dorsal in later embryos. A rostral lobe develops which is in part anterior to the stalk connecting the hypophysis to the mouth. Two lateral outpouchings arise which later form the endocranial portion. The dorsal lobe develops at the superior end of the early outpouching. An early differentiation takes place in the cells of the dorsal lobe. Buds grow out from this thickened wall and form epithelial cords between which are blood vessels and nerve fibers. Differences in the affinity for stains distinguish the rostral (and endocranial) lobes from the superior which is the chromophobic lobe in adults.

2. *Anatomy and histology*

Von Michlucho-Maclay ('68) described in Acanthias and in Scymnus a persistent connection between the mouth and the hypophysis. In his later work ('70) this investigator failed to find such a connection and believed his former observations to be incorrect.

Müller ('71) found in later embryos and adults of Acanthias that the cells forming the glandular part are columnar while those toward the periphery of the cords and tubules are spindle-shaped, with a finely granular cytoplasm. He also noted the large capillaries and the connective tissue between the tubules.

Viault ('76) briefly described the hypophysis in Raia. The hypophysis is surrounded by a connective tissue sheath which sends strands into the organ carrying large capillaries between the convoluted tubules. These tubules are 0.015 to 0.007 mm.

in diameter and usually have a small lumen. They are composed of cylindrical cells. The tongue-like anterior end, he stated, was of similar structure. He was of the opinion that the glandular portion arose from the mouth.

Rohon ('79) described an hypophysis composed of tubules in selachians. It is a triangular shaped organ with a long tongue-like projection which extends forward almost to the optic chiasma. He found no cavity within it. In Acanthias and Mustelus the hypophysis is larger than in Torpedo and Scyllium.

Sanders ('86) studied the central nervous system of Scyllium and Acanthias. He stated that the hypophysis is attached to the infundibulum by a glandular tube which lies between the inferior lobes. In a drawing of the brain of Acanthias (lateral view) he figured the hypophysis as lying caudal to the inferior lobes of the mesencephalon.

In 1892 Edinger described the mid-brain region. In Torpedo and Scyllium he found the hypophysis composed of tubules and cords of epithelium, among which are many blood vessels. He noted nerve fibers extending from the infundibular region ventralward into the hypophysis.

Haller ('96) described in detail the structure of the hypophysis of a 20 cm. Mustelus. He stated that in the adult the glands of the head (superior) portion are tubular. Secretion and cell detritus are found in the lumina. He also described an opening in the base of the anterior lobe. This is found in the thin portion of the floor in the position of the original connection with the mouth, and connects the cavity of the hypophysis with the subdural space.

Sterzi ('04) described the hypophysis in several selachians. He found the anterior part flattened dorso-ventrally, extending almost to the optic chiasma and containing a large cavity. The posterior part is separated from the remainder by connective tissue. A slender canal connects this to the anterior portion. The superior part is formed of anastomosing cords interlacing with sinusoids. The cords are formed of a peripheral layer of columnar cells and an inner mass of polyhedral ones. The nuclei of the outer zone are rich in chromatin. The polyhedral cells contain small spherical nuclei. The cytoplasm is granular and stains deeply.

Pettit ('06) made a study of the hypophysis in Centroscymnus. He mentioned posterior, median and anterior parts, all communicating. He found ramifying cords or tubules surrounded by sinusoids.

Burckhardt ('07, '11) in his work on the central nervous system of selachians described, incidentally, the hypophysis in Scymnus. He recognized a terminal, a median and a posterior lobe. Apparently he included under his division of median lobe, the caudal end of the anterior lobe of Sterzi, or it may be that the hypophysis in Scymnus is quite different from that found in other forms.

Joris ('08, '09) described the dorsal lobe in Spinax and Mustelus as formed in part from the hypophyseal evagination and in part from the infundibulum. The cell cords arise from the hypophysis while neuroglia and nerve fibres from the infundibular region grow in between these.

In 1909 Sterzi in his comprehensive work on the central nervous system of selachians, gave a clear description of the hypophysis. According to this description the hypophysis is composed of perimeningeal and endocranial parts. The perimeningeal portion is further subdivided into a dorsal and a rostral lobe. The dorsal lobe has many columns from its dorsal surface. Among these are numerous capillaries. The rostral lobe is a long flattened part extending almost to the optic chiasma. The endocranial part is composed of two sacs connected medially to one canal which leads to the anterior or rostral lobe. The cords and tubules of the dorsal lobe anastomose forming a network in which is a rich vascular supply. These are primarily attached by means of connective tissue to the base of the brain or vascular sac. The structure of these cords, Sterzi had described before ('04). The dorsal lobe, because it stained so lightly he termed the chromophobic portion. The rostral lobe has tubules extending from its ventral walls. The ventral wall has a distinct median ventral furrow and the tubules of either side remain separated. The distal ends of the tubules are solid and by means of branches anastomose with the cords of the other tubules. The arrangement of the cells here is the same as that in the dorsal lobe. This portion stains more deeply, the capillaries are smaller and less numerous. The endocranial portion in embryos is a sac with folded walls. In the adult, many tubules and cords have developed. In the main this resembles the rostral portion of the perimeningeal part.

Tilney ('11) in his studies on the comparative histology of the hypophysis, stated that in Acanthias there is a distal epithelial portion made up of parallel cell columns. The cells are deeply acidophilic with only a few faintly-staining acidophilic ones present. In the juxta-neural part the cells are larger, usually irregularly disposed, but forming some acini. However, they take the basic stains very markedly. He found this portion less vascular than the distal part. Tilney has homologized the different portions of the hypophysis in the various vertebrate groups. The distal epithelial portion of selachians corresponds to the intermediate lobe of mammals.

In 1913 Stendell made a comparative study of the hypophysis. He described intermediate and main lobes in Heptanchus. In the main lobe he described the peripheral cells of the tubules as of an acidophilic nature, while those toward the center are basophilic or neutral. Stendell has homologized the parts found in the hypophysis of selachians with those in the higher forms of vertebrates. His homology agrees with that of Tilney, i.e., the intermediate lobe of Heptanchus is homologous with the same portion in the higher vertebrates, being in Heptanchus very large and becoming smaller in the higher forms. The ventral sac of the main lobe is not found in higher forms.

There have been many terms used in the descriptions of the different parts of the elasmobranch hypophysis. A comparison with figure 1, which is a drawing of a model of the hypophysis of a pup, and a table of the terms employed may serve to explain the terms used in reviewing the literature.

TABLE 1

Terms used by the various investigators for the different parts of the elasmobranch hypophysis

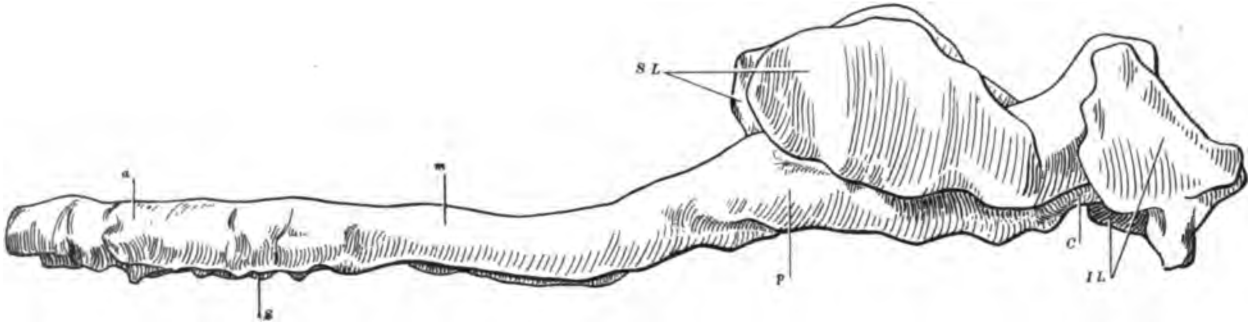
OBSERVER	MATERIAL	REGIONS OF THE HYPOPHYSIS			
		Anterior	Inferior	Superior	Special portions
Müller ('71)	Scymnus, Acanthias	Anterior part (secondary)	Posterior (main part)		
Haller ('96)	Mustelus	Superior sac	Inferior sac	Head	
Rossi ('02)	Torpedo	Median lobe; antero-median diverticulum	Antero-lateral diverticulum	Terminal lobe	Lateral lobes
Sterzi ('04)	Mustelus, Acanthias	Anterior portion	Posterior portion	Superior portion	
Pettit ('06)	Centro-scymnus	Anterior	Median	Posterior	
Burckhardt ('07)	Scymnus	Terminal lobe	Posterior lobe	Median lobe	
Gentes ('07)	Torpedo	Superior sac; anterior two-thirds	Inferior sac	Superior sac; posterior one-third	
Joris ('08)	Mustelus		Posterior	Dorsal	
Sterzi ('09)	Acanthias	Perimeningeal portion; rostral lobe	Endocranial portion	Perimeningeal portion; dorsal lobe	
Tilney ('11)	Acanthias	Juxta-neural part		Distal part	
Stendell ('13)	Heptanchus	Main lobe	Ventral sac of main lobe	Intermediate lobe	
Sterzi, Gentes, et al.		Chromophilic portion		Chromophobic portion	

MORPHOLOGY AND MORPHOGENESIS

1. *Description of the hypophysis in the pup stage*

A description of the hypophysis in the pup stage may make clear the different parts of this organ. The formation of tubules has begun and the main portions or lobes are well formed at this time.

The anterior lobe is a tongue-like process with two somewhat wider extremities; these are connected by a more slender middle part which is less than one-third of the entire length. There is a deep sulcus in the median ventral wall which extends from about the middle of the posterior extremity to near the end of the anterior extremity (fig. 1). Several other more or less regular furrows occur in the ventral surface of the anterior extremity. Two lateral constrictions separate the middle portion from the some-



· Fig. 1 Left lateral view of a reconstruction of the hypophysis of an Acanthias pup. × 50. *a*, *p*, anterior and posterior extremities of the anterior lobe; *C*, interhypophyseal canal; *IL*, inferior lobe; *m*, mid part of anterior lobe; *S*, median ventral sulcus; *SL*, superior lobe.

what more dorsal posterior extremity. These extend as furrows posteriorly from the lateral dorsal side of the caudal end of the middle part (fig. 25). As just stated, the posterior extremity is somewhat dorsal to the other parts of the anterior lobe and is directly ventral to the superior lobe. Two deep horizontal furrows constrict the connection between the posterior extremity and the superior lobe.

The superior lobe is just below the saccus vasculosus to which it is closely attached, and is almost three times as wide as the posterior extremity of the anterior lobe (fig. 25). Its median part is about as long as the posterior extremity of the anterior lobe, but projects beyond it caudally and laterally. The caudal end extends somewhat dorsally. The lateral parts, or wings, of the superior lobe project somewhat upward and forward.

The inferior sacs extend laterally from a constricted median connection. They are much smaller than the wings of the superior lobe, but, viewed from above, give the appearance of two lesser caudal wings. From their median connection a short slender canal extends in a slightly oblique direction upward and forward to connect with the anterior lobe (fig. 25).

Fig. 2 Sagittal section of the hypophysis of an 8 mm. embryo. × 40 (H. E.C. 210). *H*, hypophysis; *I*, infundibulum; *Mo*, epithelium of mouth; *Mm*, median mass connecting the premandibular somites; *N*, notochord; *Po*, post optic groove.

Fig. 3 Sagittal section of the hypophysis of a 15 mm. embryo. × 40 (H.E.C. 228). For abbreviations, see figure 2.

Fig. 4 Sagittal section of the hypophysis of a 19 mm. embryo. × 40 (H.E.C. 138). For abbreviations, see figure 2.

Fig. 5 Sagittal section of the hypophysis of a 22 mm. embryo. × 40 (H.E.C. 231). *AL*, anterior lobe; *Med*, median connection of inferior lobes; *SL*, superior lobe; other abbreviations as in figure 2.

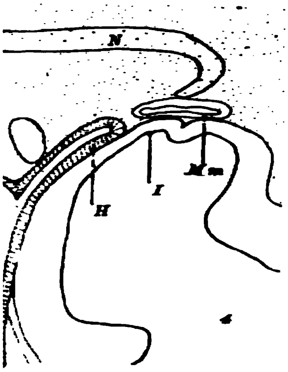
Fig. 6 Sagittal section of the hypophysis of a 28 mm. embryo. × 40 (H.E.C. 234). *St*, hypophyseal stalk; other abbreviations as in figure 5.

Fig. 7 Sagittal section of the hypophysis of a 34 mm. embryo. × 40 (H.E.C. 362). For abbreviations see figure 6.

Fig. 8 Sagittal section of the hypophysis of a 40 mm. embryo. × 40 (H.E.C. 370). *P*, parachordal plate; *T*, trabeculae; other abbreviations as in figure 5.

Fig. 9 Sagittal section of the hypophysis of a 50 mm. embryo. × 40 (H.E.C. 444). *C*, interhypophyseal canal; for other abbreviations, see figure 8.

Fig. 10 Sagittal section of the hypophysis of a pup, (reconstructed from transverse sections). × 40. For abbreviations, see figure 8.



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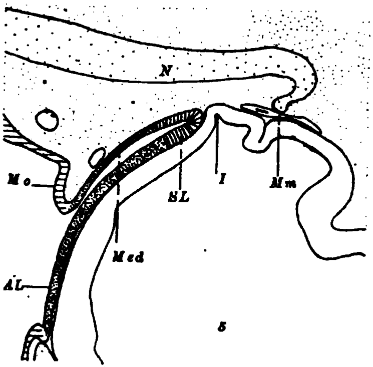

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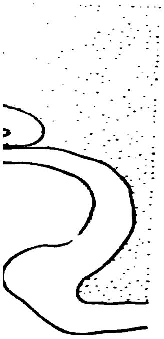

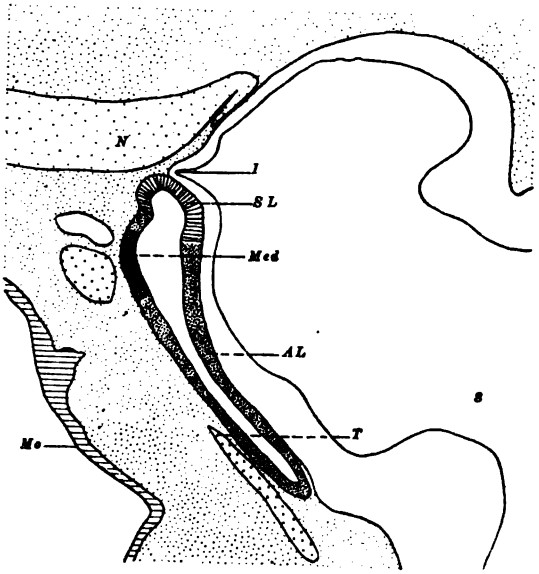

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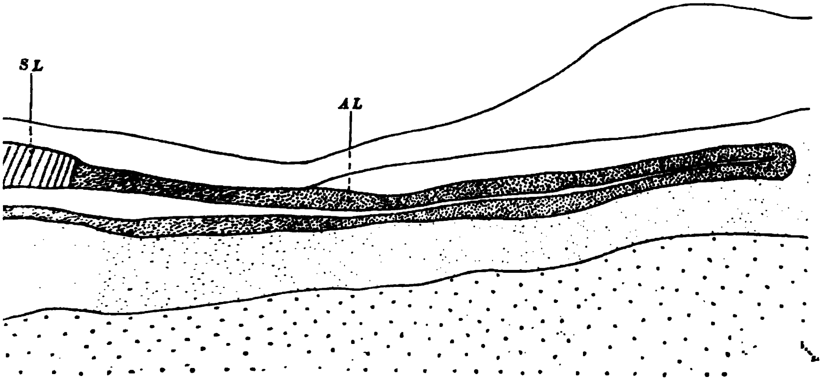

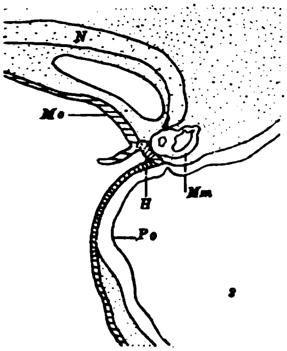
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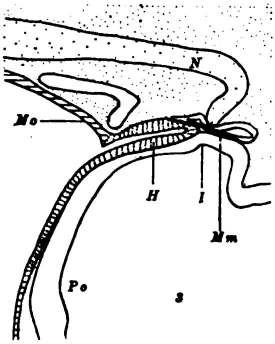
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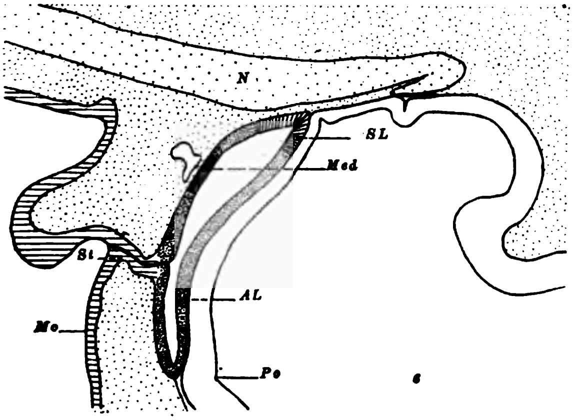
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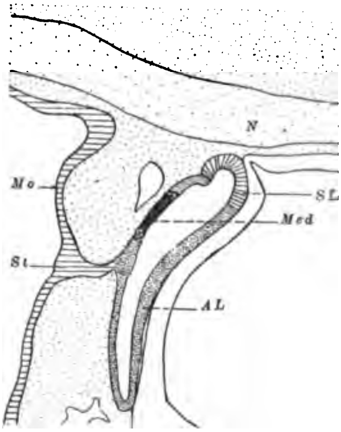
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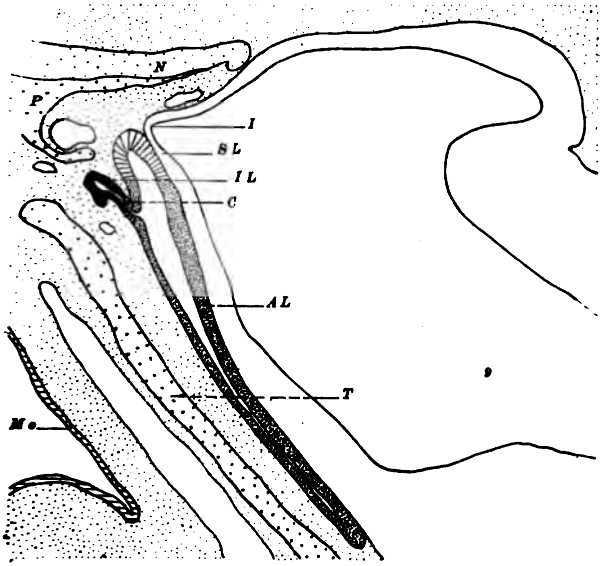
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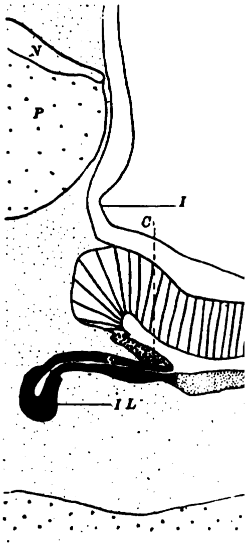
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The position of the adult hypophysis has been described by Sterzi ('09) and others. The long tongue-like anterior lobe lies on the median ventral wall of the inferior lobes of the brain. Its anterior end extends forward almost to the optic chiasma. The superior part is placed posterior to this and in a more dorsal plane. It extends as far caudally as the saccus vasculosus. The inferior sacs of Squalus do not extend as far ventrally as has been described for other selachians. But they are ventral to the superior lobe and extend farther caudalward (fig. 10). From their middle connection a slender canal joins them to the ventral side of the caudal end of the anterior lobe.

2. *Early development of the hypophysis*

Recent work on the development of the hypophysis in elasmobranchs shows that it arises at an earlier period than was formerly believed. Hoffmann ('96) stated that the position of the future hypophysis is well marked in Acanthias embryos of 15 somites but that there is no indication of an evagination even in 8 mm. (50 somites) and 10 mm. embryos. Haller ('96) began his description of the hypophysis in Mustelus in embryos 22 mm. long. At that time, the hypophysis is already a distinct outpouching. More recently Johnston ('09) briefly described the earliest formation of the hypophysis in Acanthias. In an embryo of 24 somites the ectoderm from which the hypophysis develops is readily recognized. He stated that a short anterior lobe, developing later, extends toward the optic chiasma. Also, that the posterior part crowds between the brain and the median mass connecting the premandibular somites. Scammon ('11) mentioned a thickened hypophyseal plate in a 5.2 mm. embryo and a beginning evagination in a 6.2 mm. embryo (50–51 somites).

A median sagittal section of an Acanthias embryo 8 mm. in length[3] is shown in figure 2. The anterior superior end is partially insinuated between the brain and the median mass connecting the premandibular somites, as had been noted by Johnston ('09) in about the same stage. During the time that the anterior part of the hypophyseal evagination is growing between the brain and the median mass connecting the premandibular somites, the posterior portion forms a more or less prominent ridge caudal to the connecting mass of the somites. A model of such an embryo shows as an evagination, the end of which is grooved transversely by the median mass connecting the premandibular somites (fig. 2). This groove may be quite prominent even in 11 to 12 mm. embryos and evidences of it are usually to be found at that time.

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[3] In the description of the figures 'H. E. C.' has reference to the embryos of the Harvard Embryological Collection used for this study.

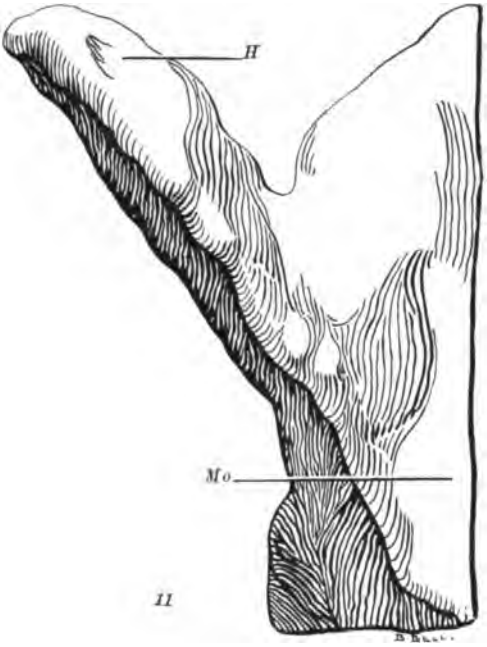
In 8 mm. embryos as well as in younger ones the anterior arm of the hypophyseal outpouching shows differentiation as far forward as the postoptic recess (fig. 2). Scammon ('11) stated that the notochord comes into contact with the early hypophyseal outpouching, and Sterzi ('12) has shown such a contact in Mustelus of 8 mm. (fig. 449). No such contact has been observed in this study of Acanthias.

A mid-sagittal section of a 15 mm. embryo shows that the superior end of the hypophysis has now grown well between the brain and the median mass connecting the premandibular somites. The evagination is more marked and the anterior arm is longer than in younger stages (fig. 3).

A model of the hypophysis of an Acanthias embryo 19 mm. in length is shown in figure 11; this view is taken from the left lateral side. The hypophysis at this stage is an anteriorly and dorsally directed outpouching, concave on its ventral surface where it lies in close relation to the diencephalon (fig. 18). Its superior anterior end extends to the infundibular recess. The opening from the pharynx into the hypophysis is small. A sagittal section at about the median line (fig. 4) shows that the thickened anterior (ventral) wall of the hypophysis extends forward as far as the postoptic groove as Johnston ('09) has figured it.

Fig. 11 Left lateral view of a reconstruction of the hypophysis of a 19 mm. embryo. × 130. *Mo*, lining of mouth; *H*, hypophysis.

Fig. 12 Left lateral view of a reconstruction of the hypophysis of a 21 mm. embryo. × 100. *a*, anlage of anterior end of hypophysis; *H*, hypophysis; *IL*, anlage of the inferior lobes; *Mo*, lining of mouth.



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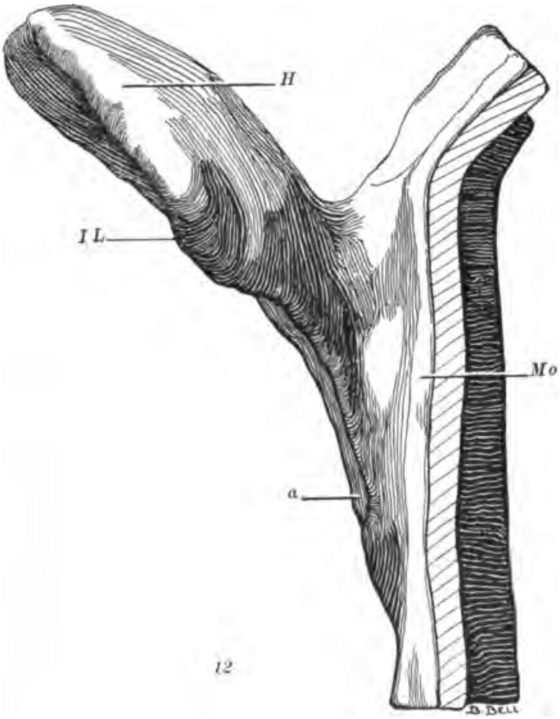

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Several changes have taken place in the hypophysis of a 21 mm. embryo. It is still concave, both laterally and dorso-ventrally in its ventro-anterior surface (fig. 12). The thickened anterior wall of the hypophysis, reaching almost to the preoptic groove, is now distinctly evaginated. Scammon ('11) mentioned this closing off of the anterior part in a 20.6 mm. embryo and Sterzi ('12) described the formation of this 'rostral diverticulum' in 20 to 24 mm. embryos. The lateral side of the anterior outpouching is sharply demarcated by the formation of the stalk connecting the hypophysis to the mouth (fig. 19). The anterior end at this stage is almost half as wide as the posterior, from which most of the hypophysis is developed. The opening from the mouth into the early anlage is located as before, but is smaller now. A view of a model from the oral side shows that the constriction of the front and lateral sides of the anterior end of the anterior lobe has begun.

In a 22 mm. embryo the hypophysis (fig. 13) is not as concave as in younger stages. The anterior part shows laterally more marked constriction from the stalk which connects it to the buccal cavity. The anterior end is also markedly constricted. The opening into the hypophysis extends now from this anterior constriction to the posterior (caudal) margin of the opening into the first outpouching. The opening into the first evagination is very small and connects the pouch with the stalk (fig. 5). The posterior end is wider transversely than before. On its dorso-lateral surfaces are small ridges (fig. 13), the anlagen of the inferior sacs. On the ventral surface of the posterior part are two slight lateral furrows which are beginning to separate the inferior sacs from Rathke's pouch (fig. 20). These furrows are present in a 20.6 mm. embryo, as Scammon ('11) stated. The lateral pouches or inferior sacs appear as dilated cavities at either side. The grooves or furrows are as yet shallow and indistinct. In an 18 mm. embryo, Scammon ('11) noted the beginning of the division, by slight furrows, of the posterior portion into a median and two lateral parts. Some embryos do indicate the beginning division of the inferior sacs about that time but the furrows are not as prominent as the dilated cavities.

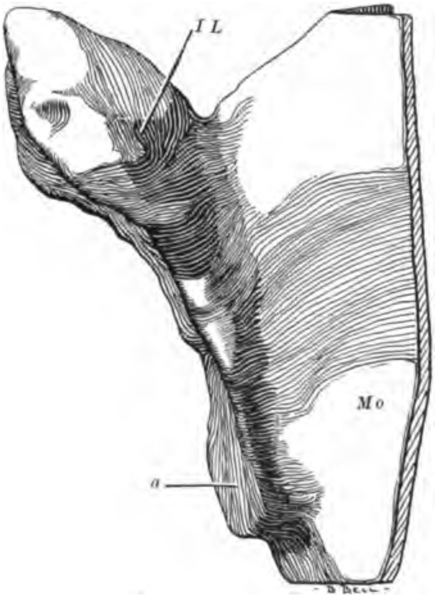


Fig. 13 Left lateral view of a reconstruction of the hypophysis of a 22 mm. embryo. × 100. For abbreviations, see figure 12.

In a model of a 28 mm. embryo (fig. 14) the entire anterior portion is well closed off. It extends anteriorly almost to the postoptic recess. From its caudal surface a slender stalk connects the hypophysis with the mouth (fig. 6). The ventral end of the anterior lobe is somewhat wider than the mid part which connects it with the very much wider posterior portion or Rathke's pouch (fig. 21). Distinct furrows on the anterior (ventral) side partially separate the lateral anlagen of the inferior sacs from the original Rathke's pouch. A slight ridge on the caudal (superior) surface joins the two inferior sacs (fig. 14). In a median sagittal section (fig. 6) a shallow groove marks the connection between them. This connection is dorsal (caudal) to the stalk joining the hypophysis to the mouth. On the lateral dorsal surface at the posterior end of Rathke's pouch two very slight outpouchings indicate the position of the developing superior lobe (fig. 14).

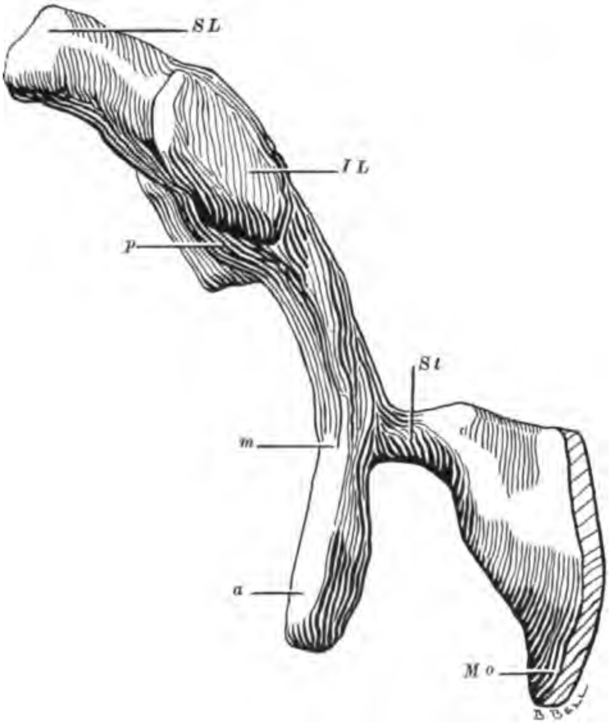


Fig. 14 Left lateral view of a reconstruction of the hypophysis of a 28 mm. embryo. × 100. *a*, *p*, anterior and posterior extremities of anterior lobe; *IL*, inferior lobe; *m*, middle part of anterior lobe; *Mo*, wall of mouth; *St*, hypophyseal stalk; *SL*, anlage of superior lobe.

3. Later development of the hypophysis

All the main outpouchings of the hypophysis are present in the 28 mm. embryo. The inferior lobes are quite prominently marked off, the anterior lobe shows two widened extremities and the superior lobe has just begun to evaginate. The anterior end of the hypophysis in a 33 mm. embryo is curved slightly forward. The anterior lobe is not so markedly concave antero-posteriorly as in earlier stages (fig. 7). The ventral (anterior) furrows separating the inferior lobes from the posterior medial portion, are now quite deep (fig. 15). On the caudal side a distinct ridge extending between the inferior lobes indicates their

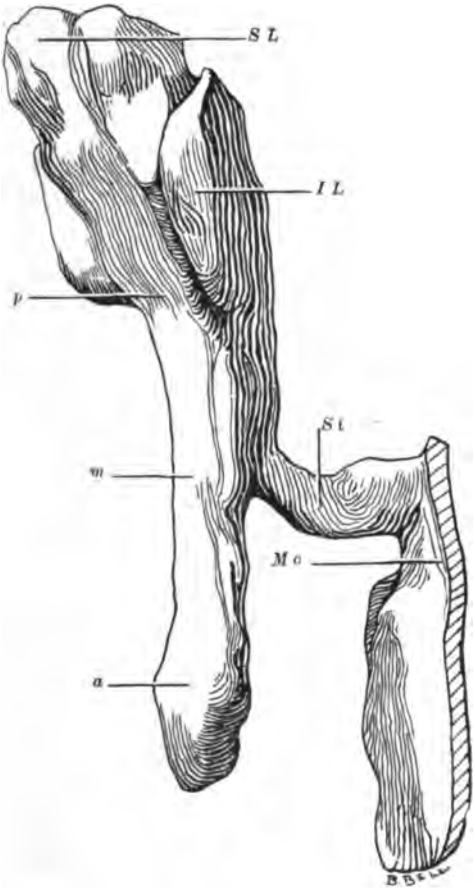


Fig. 15 Left lateral view of reconstruction of the hypophysis of a 33 mm. embryo. × 100. For abbreviations, see figure 14.

early connection with each other. On the posterior tip of the hypophysis two lateral grooves mark the beginning constriction of the superior lobe (fig. 22), the outpouching of which was seen in the previous stage. The stalk connecting the hypophysis to the pharynx is smaller than in earlier stages.

The anterior lobe of the hypophysis, particularly its anterior part, has increased in length in a 40 mm. embryo. The stalk joining the hypophysis with the mouth has disappeared, with

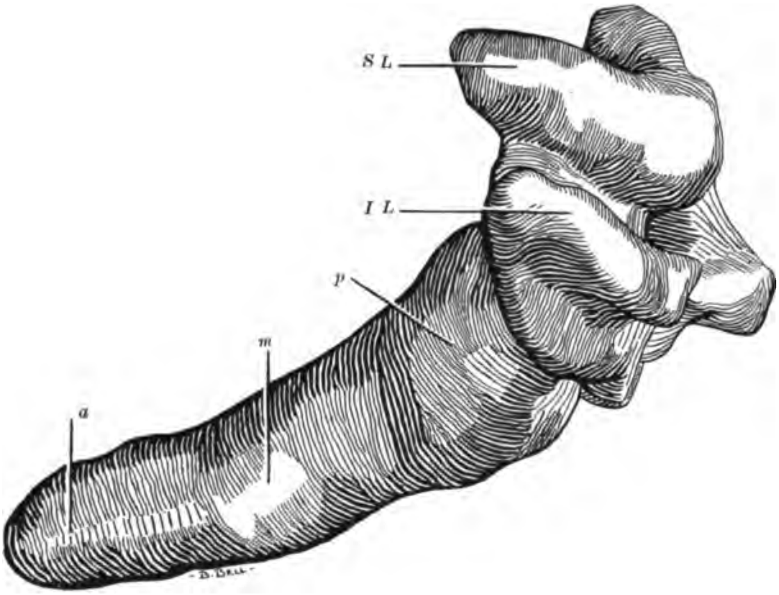


Fig. 16 Left lateral view of a reconstruction of the hypophysis of a 48 mm. embryo. × 100. For abbreviations, see figure 14.

the exception of a cone-shaped mass of cells connected with the oral epithelium, and an irregular area in the floor of the hypophysis which represents the remains of the former attachment (fig. 8). The furrow uniting the two inferior lobes across the middle line is more marked, and the superior lobe also is prominent. The anterior end of the hypophysis now extends forward and downward. The straightening out of the head bend in the development of the embryo has probably helped to bring about this change.

More marked changes have taken place in a 48 mm. embryo (fig. 16). The anterior end of the anterior lobe is wider than in earlier stages. A short and narrow middle part connects this portion with the caudal extremity which is considerably wider (fig. 23). The inferior lobes are attached to the, now, ventral (caudal) side of this part. The ridge connecting the two inferior lobes has become very pronounced but still opens widely into the anterior lobe (fig. 16). The furrows separating the inferior lobes from the anterior are much deeper and wider. The inferior lobes have enlarged in their dorso-ventral and in their transverse diameters, and they extend laterally beyond the posterior extremity of the anterior lobe. Marked development has taken place in the superior lobe. The lateral furrows separating it from the anterior one are deeper, and the cranio-caudal length of the lobe has increased so that there is a projection caudally beyond the anterior lobe. The antero-lateral ends of the superior lobe have grown forward.

The median connection of the inferior lobes is constricted from the posterior (ventral) part of the anterior lobe in a 50 mm. embryo. The inferior lobes are directly ventral to the superior lobe. There remains a short slender tube in the mid-line connecting the inferior lobes to the anterior (fig. 9). The duct connecting them to the anterior lobe extends almost straight anteriorly.

A median sagittal section of an 86 mm. embryo (fig. 27, G) shows an increase in the length of the hypophysis. The inferior lobes lie more caudally and the duct joining them to the anterior lobe is longer.

In a 95 mm. embryo the anterior lobe has increased greatly in length (fig. 17). A median ventral sulcus has appeared and the anterior third of this lobe is quite wide. A middle narrow portion, almost circular in cross section, connects the anterior extremity to a wider posterior end (fig. 24). The caudal extremity is connected dorsally with the superior lobe. The inferior lobes are continuous across the median line. The connection between the inferior lobes and the anterior one is a small tube which extends almost straight forward to join the inferior surface of the caudal end of the anterior lobe just below where this opens into the superior one (fig. 10). The inferior lobes have enlarged in their cranio-caudal axis. The lateral parts of the superior lobe have increased in their cranio-caudal diameters and extend forward beyond the median part. The latter has grown caudalward and lies just dorsal to the tube joining the inferior and anterior lobes.

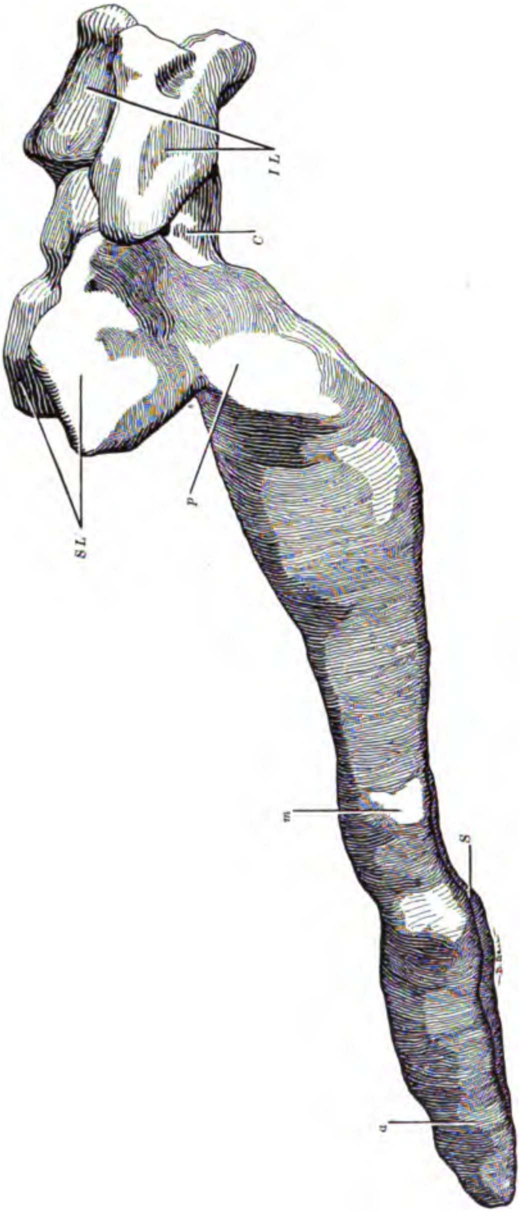
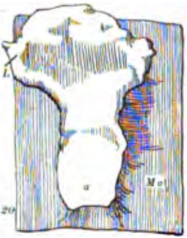


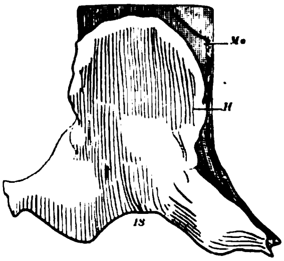
Fig. 17 Left lateral view of a reconstruction of the hypophysis of a 95 mm. embryo. × 75. *C*, interhypophyseal canal; *S*, median ventral sulcus; other abbreviations as in figure 14.

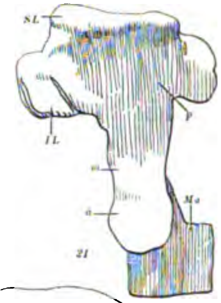
4. The hypophysis of the adult

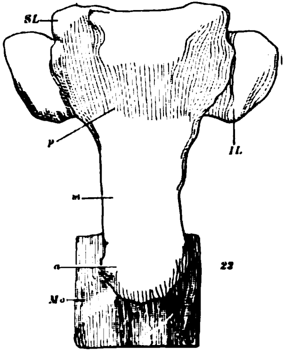
A detailed description of the pup stage has been given (p. 400) as typical of the morphology of the adult condition (fig. 1). A drawing of a dissection of the hypophysis of an adult will show the position and relation of some of the parts more clearly (fig. 26). The anterior end extends almost to the optic chiasma, as noted by Sterzi and others. From a study of the models of the 'pup' stage and of sections of adults it appears that the middle part of the anterior lobe is more than a mere constriction separating a larger rostral from a smaller caudal part as Sterzi ('09) described. In the pup this middle part is only a little shorter than either extremity, while in the adults it is much shorter. It is, however, distinctly marked. In some cases there are cystic outgrowths from the floor of this part; in others the walls and floor are quite regular and therefore are distinctly different from either extremity. The middle part is smaller than either extremity in its dorso-ventral and lateral diameters in pups (figs. 24, 25) and in adults, but the changes in diameter from either end to the middle part in adults takes place gradually and the parts are not so definitely marked, except in those cases in which no glandular outgrowths occur from the middle region which is much more prominent in sections and in wax reconstructions than is shown in dissections. The superior lobe projects some distance laterally on the ventral side of the vascular sac. It is difficult to make out the lateral limits of this part in the dissected specimen, as it seems to be continuous with the ventral surface of the vascular sac (fig. 26). In transverse sections, however, the lateral extent of the wings of the superior lobe is clear. The superior lobe is convex dorsally and closely attached to the saccus vasculosus. The latter dips down on either side of the caudal end of the anterior lobe and so partially separates the lateral wings of the superior from the anterior lobe.

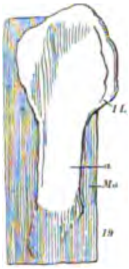
The inferior lobes no longer resemble the sac-like structures with cystic outpouchings of the pup stage. Both superior and inferior walls have developed a mass of tubular-like glands. None of these was observed extending cranialward, as described by

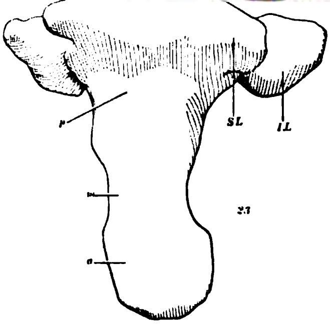


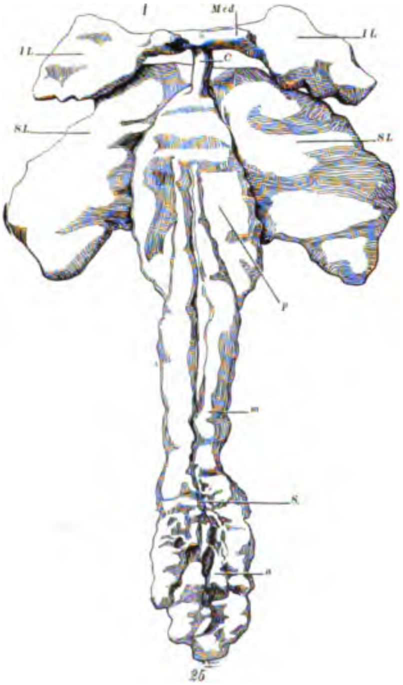


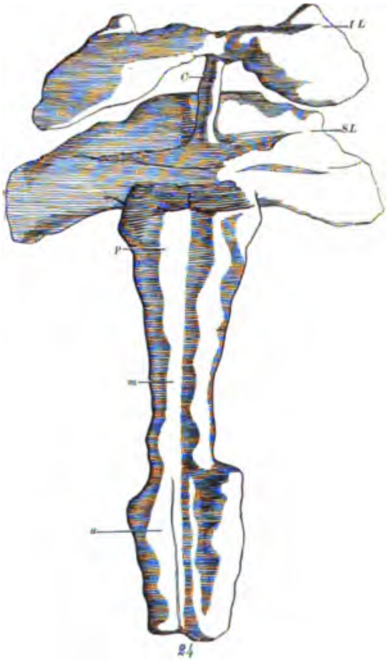












Figs. 18–20 Antero-ventral views of the reconstructions shown in figures 11 to 13. Figure 18, × 50; figures 19, 20, × 40.

Figs. 21–23 Anterior views of the reconstruction shown in figures 14 to 16. × 40.

Figs. 24–25 Ventral views of the reconstructions shown in figures 17 and 1. Figure 24, × 40; figure 25, × 25. For abbreviations, see figure 1.

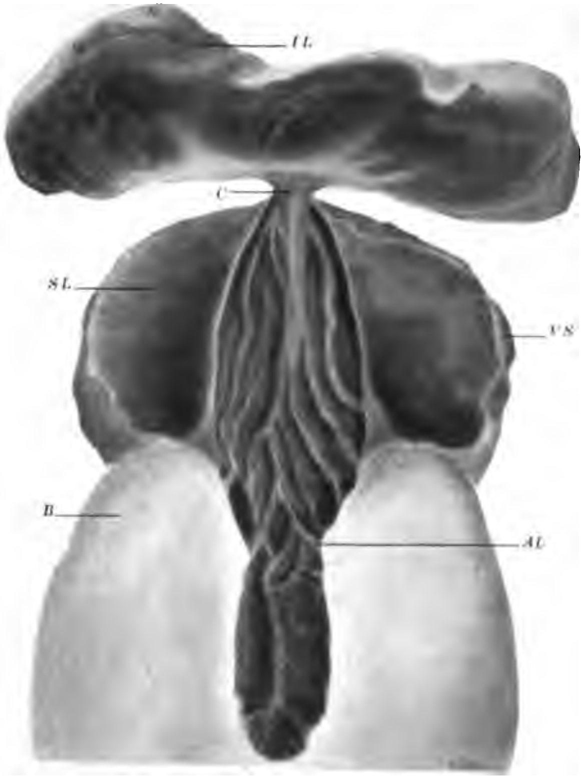


Fig. 26 Drawing of a dissection of the hypophysis of an adult Acanthias from ventral side. × 20. *AL*, anterior lobe; *B*, inferior lobes of the brain; *C*, interhypophyseal canal; *IL*, inferior lobes of the hypophysis; *SL*, superior lobe of the hypophysis; *VS*, vascular sac.

Haller in Mustelus. From their median connection a long slender tube extends to the caudal end of the floor of the anterior lobe. As seen in figure 26, the median connecting portion of the inferior lobes may be large. In some specimens this part, as well as the lobes, shows a mass of tubules. The caudal ends of the inferior lobes are surrounded by cartilage.

The shifting of the hypophysis with reference to its position in the body and the development of different parts is brought out very clearly in figure 27. Sewertzoff ('99), in his studies on the development of the skull, made use of such a figure to show the interrelation of development of the brain and skull. He made an outline drawing of the skull and brain of an embryo, choosing an arbitrary magnification. He then made drawings of different sized embryos of such a magnification that points, corresponding to two arbitrarily chosen in the first drawing, would coincide. In figure 27 of this paper, the same scheme was adopted. The magnification of the first drawing was such as to avoid as much as possible the confusion of lines. The points chosen were the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve. All the other drawings were then made so that these two points—the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve—should coincide with those of the first drawing. The outlines of the hypophysis were then drawn, using a line between the two points as a base.

The objections to such a drawing are readily apparent. There are, of course, individual variations in the embryos. Also, these points are probably continually changing during development. For a comparative study, however, the variations can be no great objection and the points chosen are probably as reliable as any. A series of embryos from 11.5 mm. in length to the pup were drawn in this manner (fig. 27). One can see at a glance in all these stages the relative position of the hypophysis with reference to the anterior end of the notochord. Also, as was pointed out in the description of the different embryos, what is first the dorsal wall becomes in later stages the ventral, while the ventral or anterior becomes dorsal. The early superior end of the evagination shifts more and more caudalward with reference to the rest of the hypophysis, until, in the pup, the superior lobe, which develops from the superior dorsal end, is caudal in position. The inferior lobes, which develop from the sides of the superior end and are on the same horizontal plane as the superior position, take a position ventral to the superior lobe in the late embryo and adult. The furrows separating these inferior lobes, described as appearing in the 20 to 22 mm. embryos

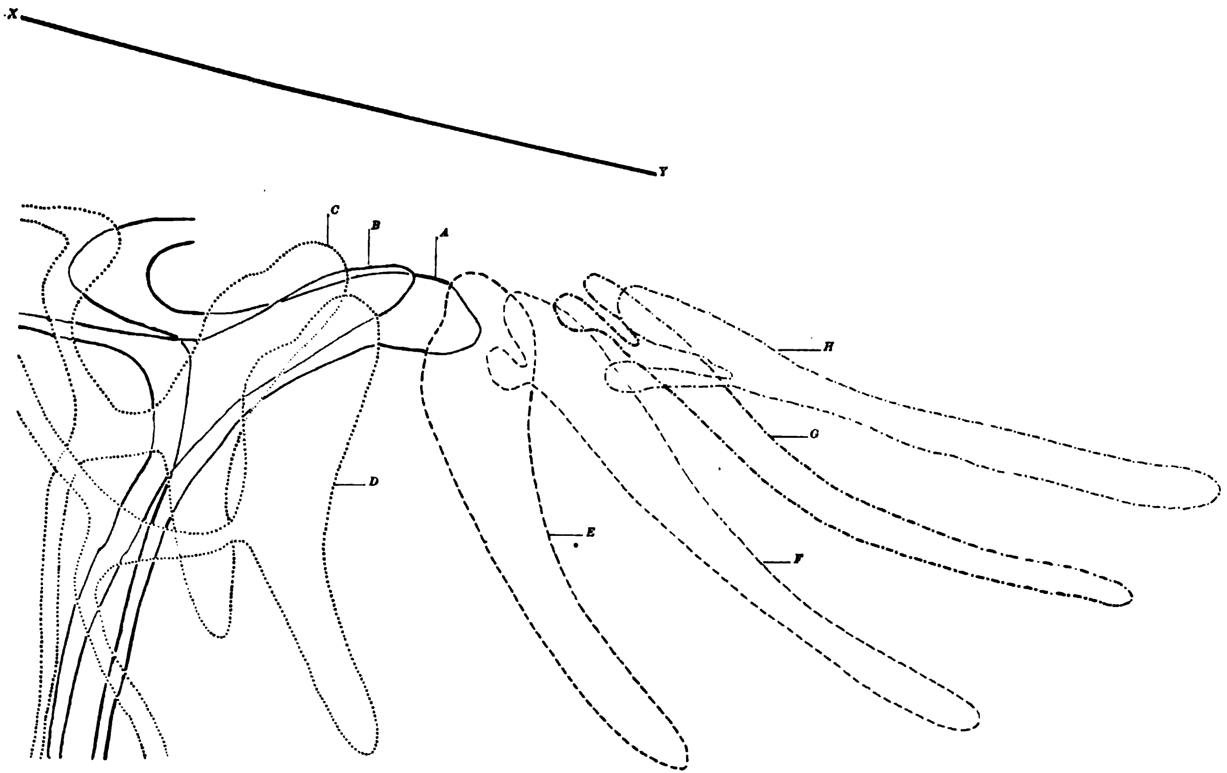


Fig. 27 Composite drawing of median sagittal sections of the hypophysis of Acanthias embryos. *A*, 11.5 mm. embryo; *B*, 18 mm.; *C*, 34 mm.; *D*, 37 mm.; *E*, 40 mm.; *F*, 50 mm.; *G*, 86 mm.; *H*, pup. *X–Y*, base line drawn from the anterior end of the notochord to the axis of the notochord at the level of the first spino-occipital nerve.

on the ventral (anterior) side of the hypophysis, are later (50 mm. embryos) on the dorsal side. The anterior lobe, first directed almost vertically, grows ventrally and later extends more and more anteriorly until it is directed horizontally (craniocaudally).

The comparative growth of the different portions is also made clear. The anterior lobe comprises all of the original outpouching and also the anterior tongue which evaginates later. The increase in length of the anterior lobe, particularly its anterior extremity, is marked. The inferior lobes, developing from the sides of the posterior portion of the early evagination, become continuous across the posterior side (34 mm. embryos) and finally constrict entirely except for a short duct connected with the anterior lobe. The inferior lobes increase greatly in size, but, in the adult, are largest transversely. The superior lobe, developed from the superior dorsal part, spreads far out transversely and later enlarges in its dorso-ventral axis.

The posterior extremity of the anterior lobe is then developed from the first outpouching. A little later (21 mm.) the part anterior or ventral to the original outgrowth evaginates, forming largely the middle narrower portion of the anterior lobe. The anterior extremity of the anterior lobe develops at the extreme anterior (ventral) end of this. The stalk connecting the hypophysis with the mouth is attached to the middle narrower portion. The connection between the inferior lobes develops caudal (dorsal) to this but arises from a part which later becomes the floor of the posterior extremity of the anterior lobe. From this description it is seen that the inferior lobes, developing from the posterior end of the hypophyseal anlage, and the superior lobe, from its extreme dorsal (anterior) end, are derived from a part of the anterior lobe. Such an explanation of the development of the parts is well borne out by a study of the models as well as of the various sections of embryos.

In comparing figures 6, 7 and 8 it is seen that the hypophyseal stalk in a 40 mm. embryo is attached nearer the caudal end of the hypophysis than it is in younger ones. This does not agree with what Haller has described for Mustelus when he found the place of attachment of the hypophyseal stalk near the anterior end. In a 90 mm. Mustelus this place of attachment can be recognized by the very thin floor, and in the adult, by the opening into the subdural space (Haller '96, figs. 12 and 40).

The cavity in the hypophysis of elasmobranchs has been variously described as barely distinguishable, slit-like and large. It *is* large in some adult specimens of Acanthias. In the anterior lobe there is a distinct increase in the size of the cavity during its development. Table 2 will show the actual increase in the depth of the cavity. The measurements here given were taken in the caudal part of the anterior extremity of the anterior lobe.

TABLE 2

Showing depth of the hypophyseal cavity

SPECIMEN	THICKNESS OF ROOF IN MICRA	DEPTH OF CAVITY IN MICRA	THICKNESS OF FLOOR IN MICRA
Embryo 34 mm.	31	37	25
50 mm.	31	13	18
86 mm.	35	12	22
Pup	56	90	50 including glandular outgrowths
Adult	56	470	480 including glandular outgrowths

The increase in size of the lumen from the pup to the adult is thus seen to be considerable. From the table and from comparison with figures 2 to 10 it is seen that the increase in size of the lumen is not gradual through all the stages. For example, in the 50 mm. embryo the lumen is actually smaller than in a 34 mm. embryo. It is only from the early pup stage on that the lumen increases to any considerable degree. The increase in size of the lumen of the inferior lobes is even more marked. The cavity in the superior lobe is never large. It is very small in a 48 mm. embryo where the lateral wings are first prominent. In a 95 mm. embryo this cavity is small, but is still distinct. The lumen of the middle portion of the superior lobe extends transversely and forward in the lateral wings of the superior lobe in pups. There is no evidence at any time of an extension of the lumen into any of the glandular columns of this part. In the adult all trace of a central cavity has disappeared, unless the small secretion spaces, to be described later, are remains of the original lumen. Occasionally there is a more or less prominent median dorsal extension of the cavity of the anterior into the superior lobe. In a very few cases this dorsally-extending cavity is continuous with a secretion space lying laterally.

Table 3 shows the increase in size of the hypophysis; also, the length of the hypophysis at the median line, the greatest width of the anterior extremity of the anterior lobe, and the greatest width of the superior lobe. The table shows there has been a constant increase in length which is rather more rapid in earlier stages. The increase in width of the superior lobe is a gradual one in the beginning. The anterior lobe, however, grows very little at first. This part doubles in width in its growth between the 22 mm. and the pup stages. It almost doubles again in its later growth.

TABLE 3

Showing increase in size of the hypophysis

SIZE OF SPECIMEN	LENGTH OF HYPOPHYSIS IN MM.	GREATEST WIDTH OF SUPERIOR LOBE IN MM.	GREATEST WIDTH OF ANTERIOR EXTREMITY IN MM.
Embryo 22 mm.	0.69		0.23
28 mm.	0.81	0.40	0.22
33 mm.	1.04	0.96	0.30
48 mm.	1.00	1.07	0.35
95 mm.	1.34	1.30	0.40
Pup	3.54	1.91	0.53
Adult	6.00	4.00	1.00

From a brief study of a few Torpedo embryos, I am inclined to believe, with Sterzi, that the lateral lobes, described in this form by Gentes ('08) and others, are comparable with the inferior lobes of Acanthias. The embryos examined show no prehypophyseal body such as Chiarugi ('98) described in this genus, nor is there anything comparable to the prehypophyseal body found in the embryos or adults of Acanthias.

HISTOLOGY AND HISTOGENESIS

1. *Histology of the adult hypophysis*

a. Anterior lobe. The deep median sulcus in the floor of the anterior lobe has been noted above. It has been observed by Sterzi and others in various selachians. The furrows noted in the floor of the anterior extremity in the pup are evidence of the beginning formation of tubules. Haller observed the tubules on the ventral wall of the anterior part in Mustelus, Gentes observed them in Torpedo, and Sterzi in Acanthias. Tilney ('11) stated that there are many vesicles in the upper and lower walls of the juxta-neural (anterior) part in Acanthias. Haller figures cyst-like glands in the roof of the anterior lobe of Mustelus. Some adult specimens of Acanthias show glandular outgrowths in the superior wall of the anterior lobe, especially in the dorso-lateral parts of the anterior extremity. As Sterzi ('09) observed, glands probably develop from the anterior lobe throughout life and, finally, even from the dorso-lateral walls and roof, and glands project ventrally from the floor of the anterior lobe. A model of some of the latter shows that branches are given off at all angles from the first large tubule extending ventrally and that secondary and tertiary outpouchings occur from the branches. The tubules anastomose among each other and with those from other glands both cranially and caudally, but not across the median sulcus with the glands of the other side. The lumina may be continuous through the anastomosing tubules. A cast of the lumina shows frequent enlarged cavities from which small openings lead to the cyst-like cavities found in some of the secondary and tertiary tubules. No such glands were observed in any other part of the anterior lobe. If any glands be present in the roof of the anterior end they are simple and cystic or acinar in character. In some specimens, probably older animals, there are large tubular glands in the floor and lateral walls and even in the roof of the caudal extremity. The floor of the middle part occasionally shows cystic glands. The walls of the tubules are two or three cells thick and the columnar cells forming them are at right angles to the surface (fig. 28). There is a periph-

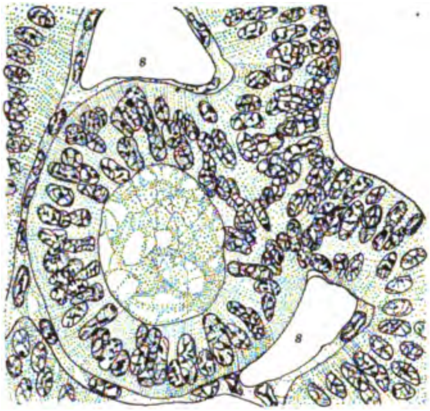


Fig. 28 Transverse section of the floor and a gland of the anterior lobe of an adult. × 400. *s*, sinusoid.

eral zone of cytoplasm which is very finely granular and contrary to the statement of Tilney, it is acidophilic. Haller observed that the peripheral zone of cytoplasm in Mustelus stained intensively with borax carmine and Stendell stated that the peripheral layer of cells is acidophilic. Sterzi ('09) found that the cytoplasm of the cells in the floor of the caudal part of the rostral lobe is not granular. However, there are glandular outgrowths here in some adults and these walls have the same character as the other glandular parts, except in the mid-ventral line where no outgrowths are found, and here the wall is composed of low epithelial cells. The nuclei are oval and crowded nearer the inner free surface. They have a finely granular chromatic network. The roof of the anterior lobe is also composed of several layers of cells. If no glands are present, the nuclei here are very irregularly placed, some of them lying parallel to the surface along the inner free side, others being placed at various angles to the surfaces. The cytoplasm is very scant. The roof comes into close relation with the overlying brain, but a thin connective tissue layer containing small blood vessels and capillaries separates them. One cannot speak of a fusion of the roof of this lobe with the brain tissue above, as noted by Tilney.

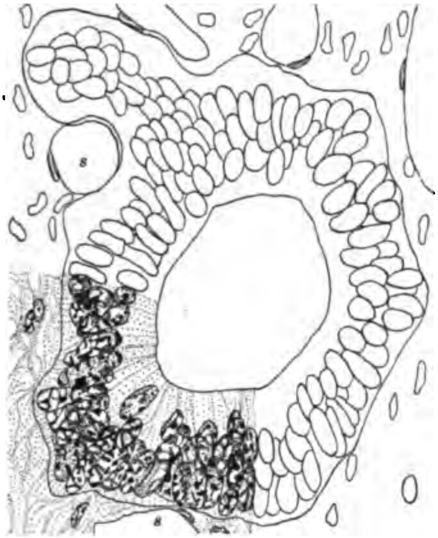


Fig. 29 Transverse section of a gland of the inferior lobe of an adult. × 400.

b. Inferior lobes. The inferior lobes are large glandular structures, from the walls of which are many tubular outgrowths. These tubules are especially numerous on the ventral surfaces of the inferior lobes although there are some on the roof also. Two or three layers of columnar cells form the walls. In this case a wider cytoplasmic zone lies along the inner free surface (fig. 29). The cytoplasm is clear and the cell membranes stand out distinctly. The cells are very faintly acidophilic. The nuclei here, as in the anterior lobe, are oval in outline, and the long axis is always at right angles to the free surfaces. The chromatin here also is in a fine network with a more or less definite layer along the nuclear membrane.

c. Superior lobe. As has been observed in all selachians, there is a great glandular outgrowth from the roof of the superior lobe. As Sterzi has noted, these glands are not tubular but solid. The floor of this portion shows no glandular outgrowth. It is made up of several layers of columnar cells. The roof is several cell-layers deep and from it many thick columns extend upward.

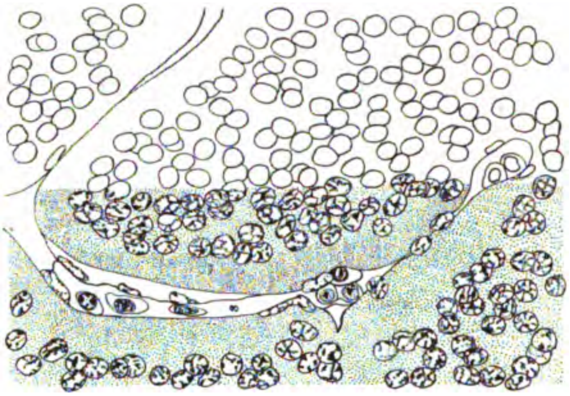


Fig. 30 Sagittal section of the glandular cords of the superior lobe of an adult. × 400.

The nuclei of the columns are spherical in shape, crowded close together in the center, and contain a light chromatin network and a nucleolus. Between these columns are numerous sinusoids. Along the periphery of the columns is a thick layer of granular cytoplasm, the granules being small and closely crowded (fig. 30). The cytoplasm frequently takes a distinctly acid stain. In some eosin-methylene blue preparations the cytoplasm of the superior lobe along the periphery of the columns is stained blue. With iron hematoxylin this same zone sometimes retains the hematoxylin longer than does the chromatin. The granules of this part often stain an intense blue with Mallory's phosphotungstic acid stain. On either side of a granular area which stains very deeply there may be a clear area where the secretion possibly may be forming or has just been given up. I cannot, therefore, agree with Tilney ('11) who finds only eosinophilic cells in the superior lobe and basophilic in the anterior lobe. Besides, as has been noted, in many cases the cells of the anterior lobe take eosin quite as readily. Stendell ('13) thought that the division of chromophobic and chromophilic portions as described by Sterzi and others might not be true in all cases. In the adult Acanthias, it seems to me this distinction cannot be sharply drawn, at least not with all stains. The cytoplasm of the superior lobe stains readily, and indeed, sometimes more deeply than that of the anterior lobe. The nuclei, on the other hand stain less deeply than those of the anterior lobe. Those of the inferior lobe frequently stain very lightly. Sterzi has stated that the cytoplasm of the superior lobe stains with difficulty.

d. Connective tissues, blood vessels and nerves. In very small embryos there are only occasional connective tissue cells lying between the hypophysis and the brain and vascular sac above, and these cells are along the dorso-lateral walls. Beginning in 33 mm. embryos, however, there is a thin layer of mesenchyma between the dorsal (posterior) end of the hypophysis and the anlage of the vascular sac. In 50 mm. embryos small blood vessels are found between the superior lobe and the saccus vasculosus (fig. 37). At this time, also, a thin layer of mesenchyma separates the hypophysis from the brain floor. In the superior lobe of the adult occasional small strands of connective tissue are found in the center of the core of nuclei of the columns.

They anastomose with the connective tissue between the columns but have no cytoplasmic zone bordering them as do the larger, more vascular connective tissue strands between the columns.

In the pup, there are small capillaries in the connective tissue over the anterior lobe of the hypophysis. On the ventral side there is considerable connective tissue between the developing tubules. The few capillaries here are not large. The capillaries between the columns of the dorsal lobe are large and numerous. These capillaries or sinusoids are to be found in the interstices between the cell columns and also between the ends of the columns and the overlying vascular sac. In the adult the capillaries over the anterior lobe are somewhat larger. There has been some increase in the size and number of the capillaries between the tubules of the anterior lobe and the same is true in the superior lobe between the cell columns.

Nerves have been described in the hypophysis by Edinger ('92), Sterzi ('09) and others. Sterzi described the floor of the brain in the hypophyseal area (above the superior lobe of the hypophysis) as composed of three layers, of which the middle was made up of nerve fibers coming from the caudal end of the inferior lobes of the brain. When these fibers reach the area above the superior lobe, numerous bundles of them go ventrally between the columns. These bundles are large and composed of large nerve fibers. According to Stendell ('12) a distinct lumen, continuous with the lumen of the vascular sac, extends into these bundles in Heptanchus. As Sterzi ('09) showed, these bundles are solid in Acanthias. Sterzi was not able to see any of the fibers ending in the cell columns. In some material stained with Mallory's phosphotungstic acid hematoxylin these fibers are well shown. I can not affirm that they do end in the cell columns between the cells as Sterzi was inclined to believe.

e. Secretions. Haller ('96) stated that the lumina of the superior lobe may contain cell detritus or a secretion. Tilney ('11) observed a colloid-like substance in the vascular sac above the superior lobe. Stendell ('13) described deep acidophilic secretion granules in the cytoplasm of the cells of the 'Zwischenlappen.' These colloid-like secretions were found in the cells lying towards the blood vessels. The adult Acanthias studied show no colloid-like secretion granules, such as Stendell found in the cytoplasm near the sinusoids in Mustelus and Scyllium. Considerable colloid-like secretion, however, is found in the tubules of the anterior lobe, also some in the main lumen of this part. The tubule drawn in figure 28 contains secretion. Some secretion was found in the tubular glands and in the main lumen of the inferior lobes. There are also many spaces in the superior lobe which are partially filled with secretion. The spaces are cylindrical in shape, sometimes as much as 20μ in diameter and 50 to 200μ long. They have no special walls, the cells and nuclei lining them appear to have been crowded aside. Frequently the inner layer of nuclei lies flat along the wall. These spaces never come in contact with the sinusoids, but are always found in the middle of the columns and are surrounded by nuclei which are crowded close together. Aresu (14) has described similar cyst-like spaces in Chimaera, containing a substance which stains lightly with basic stains. That the secretion does not fill the spaces may be due to shrinkage, as has been suggested in the case of the colloid in the thyreoid follicles. In all respects it is similar to that found in the anterior lobe. There is no direct outlet by means of which a secretion can reach the cavity of the vascular sac and thus gain entrance to the ventricles of the brain. The absence of any secretion in the vascular sac also argues against there being a pathway for the secretion to enter it. It would seem probable that another secretion, distinct from this, is formed in the lumen of the tubules. As stated in the description of the histology of the adult hypophysis, the granular cytoplasm is always found on the peripheral side of the cell cords or tubules and never on the side toward the lumen. It is probable that this secretion finds its way into the numerous capillaries along the periphery. In the formation of two secretions, therefore, the hypophysis resembles the thyreoid in some forms, and also the hypophysis in some mammals.

2. Histogenesis of the hypophysis

In 7.5 mm. embryos the walls are formed of a single layer of cells of a large cuboidal type. The cytoplasm is slightly granular and somewhat acidophilic. The nuclei are large, somewhat oval, placed near the basement membrane, and contain considerable chromatin network. Usually several nucleoli, although sometimes only one, are found near the nuclear membrane (fig. 31). Very soon the walls of the hypophysis are composed of several layers of cells. In a 13 mm. embryo the large nucleoli are no longer so prominent and, as a rule, only smaller pseudonucleoli, as observed by Sterzi, are to be seen.

a. Anterior lobe. In a 21 mm. embryo, very little of the floor of the anterior lobe is as yet present. The walls are composed of two layers of columnar cells which have a thin outer and a thick inner, slightly granular cytoplasmic zone. The large and elongately oval nuclei contain a dense chromatin network, especially in the anterior end (fig. 32). Sterzi has observed that there are some elongated nuclei in the roof of the hypophysis in which the long axes are at right angles to the surfaces, and

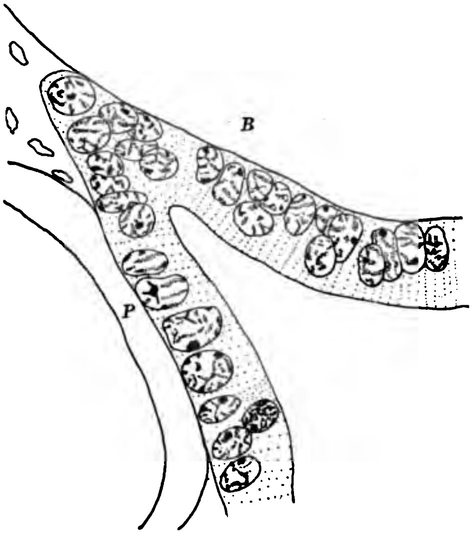


Fig. 31 Transverse section of a portion of the hypophyseal anlage of a 7.5 mm. embryo. (H.E.C. 1503). × 500. *B*, region of brain; *P*, premandibular somite.

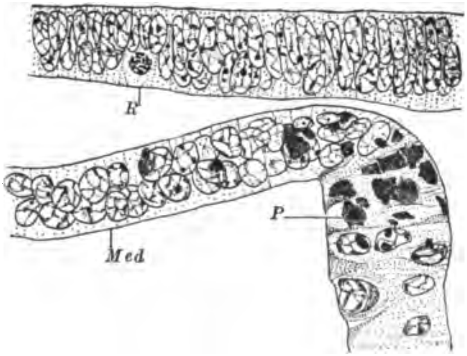


Fig. 32 Sagittal section of the roof of the anterior lobe in a 21 mm. embryo. (H.E.C. 1493). × 450. *R*, roof of anterior lobe; *Med*, medial connection of inferior lobes; *P*, pigment granules.

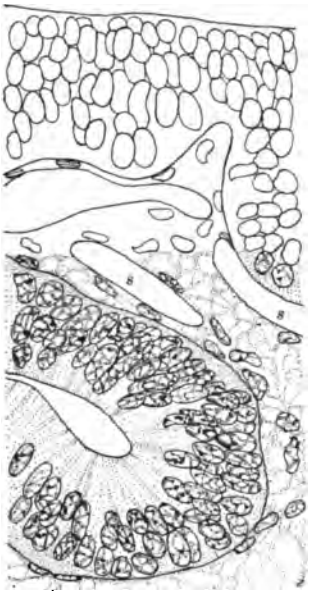


Fig. 33 Sagittal section of the floor of the anterior end and of one of the tubules of the anterior lobe of a pup. × 400.

between these are spherical shaped nuclei. He has described these in the roof of the superior lobe. These become more evident in 40 and 50 mm. embryos. In an 86 mm. embryo, the nuclei of the roof are to some extent irregular in arrangement. The cytoplasm takes orange G quite readily and hence is somewhat acidophilic. In the pup, the floor is well formed and is now the thicker wall of the anterior lobe. As shown in figure 33, it is composed of four or five layers of nuclei. The oval nuclei are crowded close to the inner free surface. There is, however, a narrow outer cytoplasmic zone which is very granular. The character of the cells of the glandular outgrowths is shown in figure 33. Here, too, there is a narrower outer rim of granular cytoplasm. A wider inner zone of non-granular cytoplasm bounds the lumina of the tubules. The nuclei, as in the floor, are oval. Usually two or three layers form the walls. The nuclei have a distinct chromatin network with occasional denser-staining chromatin masses resembling nucleoli.

b. Inferior lobes. The development of the inferior lobes as constrictions of the lateral walls of the early hypophyseal outpouching has been described above. The point where these lobes will grow together across the median line is indicated in figures 5 to 10. The character of the cells forming this part differs from the rest of the hypophyseal outpouching as early as the 21 mm. stage (fig. 32). The cytoplasm here stains less densely than that of the rest of the hypophysis. The nuclei are distinctly spherical in shape and have a very scant chromatin network. A part of this floor, immediately posterior to the hypophyseal stalk, contains a considerable amount of a granular yellowish pigment. Both Müller ('71) and Hoffmann ('96) have noted the pigment in the stalk of the hypophysis. The nuclei become more oval in older embryos. This is well shown in figure 34 which is a mid-sagittal section of the region which later forms the connection between the inferior sacs. The nuclei here are very irregularly placed. Extending caudally from the stalk are several nuclei flattened along the inner free surface. The cytoplasm stains very lightly. The pigment masses are numerous. Caudally there is a sudden transition to columnar cells of the kind found in the wall of the anterior lobe. The floor in this region is as just described until the 48 to 50 mm. stages when the inferior sacs are completely constricted from the anterior one. The pigment and the flattened nuclei are found only near the median line. Both are still present in a 41 mm. embryo.

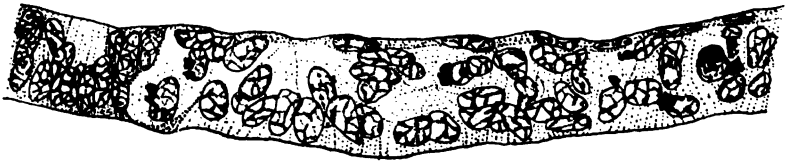


Fig. 34 Sagittal section of the floor of the hypophysis near the median plane, showing the part which connects the inferior lobes. (H.E.C. 362). × 400.

In the inferior sacs proper, however, which are formed at the lateral sides, the cells are similar to those of the floor of the anterior lobe. The outer, narrower zone of cytoplasm, as well

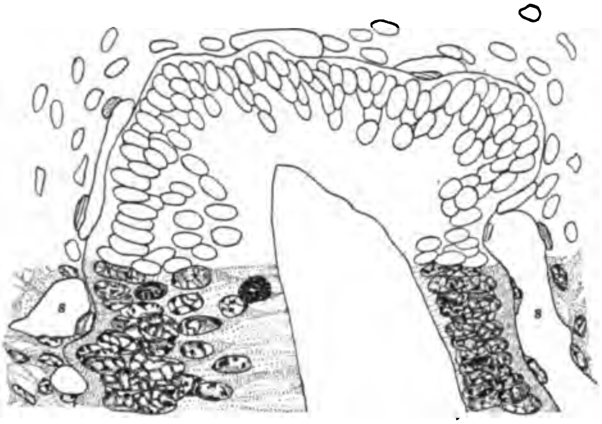
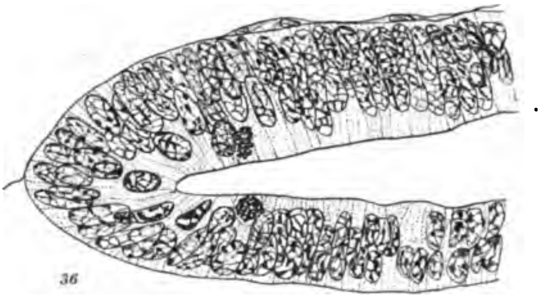


Fig. 35 Sagittal section of a portion of the inferior lobe of the hypophysis of a pup. × 400.

as the inner, wider one, stains lightly in a 50 mm. embryo. In an 86 mm. embryo the outer zone has fine granules. The cells are slightly acidophilic. The nuclei are elongately oval in outline as in the anterior lobe. In the pup many outpouchings indicate the beginning glandular development. The upper wall is three or four cell-layers thick (fig. 35). There is a wide, inner, clear-staining cytoplasmic zone. The outer narrower rim is slightly granular. The nuclei are oval in outline, as in the anterior lobe. Numerous densely staining chromatin masses are to be seen. The lower wall or floor is much thinner. It is composed of only one or two layers of cells and has a narrow, inner, cytoplasmic rim. This zone, as in the roof, stains very lightly and is non-granular. The outer rim is, however, quite granular. The nuclei are like those in the roof but contain a somewhat denser chromatin network.

c. *Superior lobe.* In a 21 mm. embryo the wall of the superior end of the hypophyseal anlage is thickest where the superior lobe later develops (fig. 36). The nuclei are large and oval and have a light chromatin network. In a 50 mm. embryo (fig. 37) the wall in this region is considerably thicker than in the 21 mm. embryo because of an increase in the number of cell layers. There



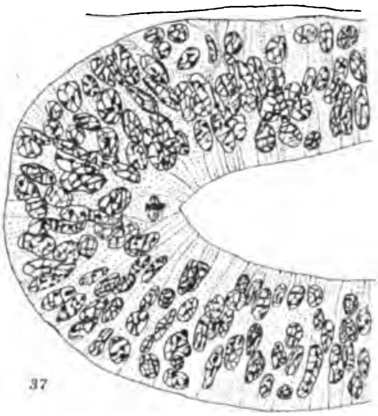
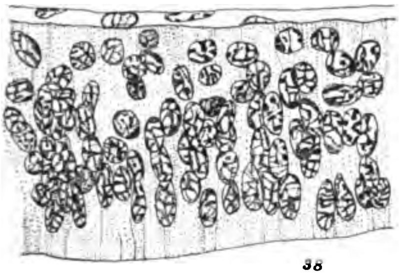


Fig. 36 Sagittal section of the superior end of the hypophysis of a 21 mm. embryo. (H.E.C. 1493). × 450.

Fig. 37 Sagittal section of the superior lobe of a 50 mm. embryo. (H.E.C. Series 444). × 450.

is an outer cytoplasmic zone which is non-granular and stains lightly. The nuclei are smaller than in the 21 mm. embryo and contain less chromatin. As Sterzi has stated, there are some spherical nuclei and some more slender oval nuclei, but no regular arrangement of these, such as Sterzi described, was observed. In a 95 mm. embryo, the roof of the superior lobe has increased in thickness (fig. 38). The nuclei are oval and contain a denser network of chromatin than is found in 50 mm. embryos. Along the periphery many of the nuclei are spherical. There is an inner zone of cytoplasm as in the 50 and 21 mm. stages, which is



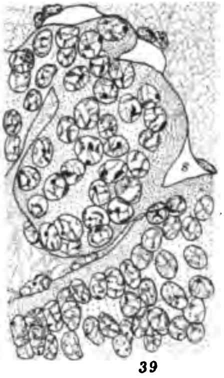


Fig. 38 Transverse section of the superior lobe of a 95 mm. embryo. (H.E.C. Series 1882). × 450.

Fig. 39 Sagittal section of the superior lobe of the hypophysis of a pup showing one of the cell columns and a portion of the roof. × 400.

non-granular. Fewer mitotic figures are to be found at this time. In the pup the roof is a little thicker than in the preceding stage. Above the roof there are many cell columns which are outgrowths from the roof proper (fig. 39). The relations of cell columns and roof to each other are shown in figure 39. The roof has a narrow outer zone of granular cytoplasm. Its nuclei are oval and have a light chromatin network. The cell columns come into close contact with the overlying floor of the vascular sac (fig. 39). Numerous capillaries and a loose connective tissue fill the spaces between the cell columns and between the roof of the hypophysis and the floor of the vascular sac. The columns have an outer, granular cytoplasmic zone. The cells are acidophilic. The nuclei are spherical in outline, have a light chromatin network and usually one or two larger chromatin masses or nucleoli.

Only a brief statement can be given at this time concerning the development of the glandular columns of the superior lobe. In the region of the superior lobe in all embryos up to the 50 mm. stage, the nuclei are elongate-oval in outline and are arranged in two or three layers (fig. 36). In a 50 mm. embryo some of the nuclei at the periphery are spherical (fig. 37). It is possible that the elongate-oval nuclei in this region in the younger embryos are changed into spherical ones. In the pup there are numerous columns extending dorsally, which consist of a central group of spherical, light-staining nuclei and a peripheral zone of cytoplasm. Until some material between the 95 mm. embryos and the pup stage is studied, it must remain a question how these cell columns are formed. It is possible that the cells with spherical nuclei arrange themselves in groups and these groups then evaginate from the roof. The scarcity of these groups in the 95 mm. embryos and in all late embryonic stages argues against such a possibility, although Sterzi's observations on the presence of groups of spherical nuclei lying between masses of cells with oval nuclei should be taken into consideration. Numerous cyst-like outpouchings are present in some adults in the anterior part of the superior lobe, or, rather, between this and the roof of the caudal extremity of the anterior lobe. Some of these outpouchings show areas of cells similar to those forming the columns of the superior lobe, interspersed with areas of cells like those of the anterior lobe. The areas of cells resembling those of the superior lobe may form the entire wall or may lie on a basement of cells resembling those forming the anterior lobe which line the cavity. This would indicate that the regions of the anterior and superior lobes are not sharply separated, or, that the cells of this region which still resemble the embryonic condition change into cell columns of the superior lobe. This need not imply, however, that the cells of the anterior and inferior lobes are of a more embryonic type, although they may be more primitive phylogenetically.

3. Development of the interhypophyseal canal

The formation of the ridge connecting the inferior lobes on the dorsal (posterior) side of the hypophyseal anlage had been described. The character of the epithelium in this region in a 21 mm. embryo, as stated above, differs from that of other parts. A ridge is prominent in a 34 mm. embryo. In a 40 mm. embryo the groove on the inside of this ridge is marked (fig. 8). In a

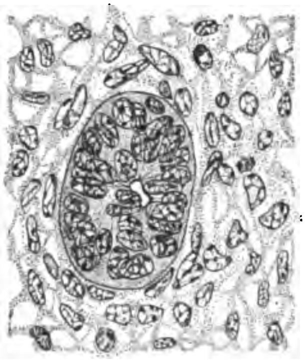
48 mm. embryo the ridge is very distinct and the connection of the lumen of this part with the lumen of the anterior lobe has become constricted. The constriction forms the narrowed connection of the inferior sacs to the anterior lobe. As previously stated, the growth of the furrows separating the inferior sacs from the lateral sides of the anterior lobe is well marked at this time. A sagittal section of the hypophysis of a 50 mm. embryo shows a short interhypophyseal canal (fig. 9). In a 95 mm. embryo the canal has lengthened considerably. The walls are composed of one or two layers of low columnar cells. In the pup the canal is longer than in the embryos but the diameter is about the same as in younger specimens. In the adult the canal has increased in length and diameter and is attached in the floor of the anterior lobe at or near its caudal end. Its other attachment is near the dorsal side of the connection between the inferior lobes. There are no tubular outgrowths from it such as Haller found in Mustelus. Table 4 shows the size of the canals at different stages. It will be seen that there is a continual increase in the length of the canal. The diameter in the older specimen is somewhat greater than in the 50 mm. embryo, although there is no great change. A distinct lumen is present. In the median line there is a well defined layer of connective tissue extending from the tip of the parachordal plate (fig. 9) forward to the floor of the anterior lobe of the hypophysis. This layer develops rapidly after the canal is formed and surrounds it. It is well defined in a 50 mm. embryo, but becomes thicker in the adult. This layer separates the inferior lobes from the rest of the hypophysis and

TABLE 4

Showing the size of the interhypophyseal canal

SIZE OF SPECIMEN	LENGTH OF CANAL IN MM.	DIAMETER OF CANAL IN MM.
Embryo of 50 mm	0.13	0.042
95 mm	0.36	0.048
Pup	0.44	0.048
Adult	0.68	0.080

makes their dissection difficult. The cells and fibers in it are arranged concentrically around the canal. In the adult, the layer of connective tissue is still prominent and extends the entire length of the canal.



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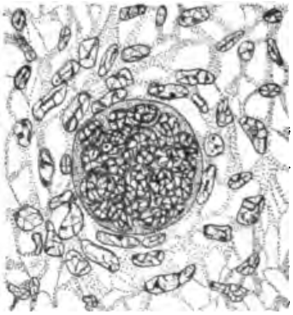

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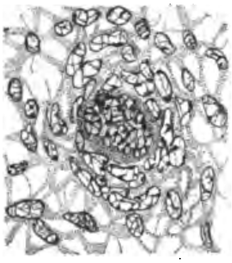
Fig. 40 Transverse section of the hypophyseal stalk of a 28 mm. embryo. (H.E.C. Series 1357). × 350.

Fig. 41 Transverse section of the hypophyseal stalk of a 33 mm. embryo. (H.E.C. Series 307). × 350.

4. Development of the hypophyseal stalk

The stalk connecting the hypophysis with the mouth is formed in 22 to 24 mm. embryos. It is present in one 21 mm. embryo which shows the anterior end constricting from the mouth. In these stages it is oval in cross-section, the lumen is very large, and its walls are formed of a layer of low columnar cells, the nuclei of which are somewhat elongated and contain considerable chromatin. In the posterior (superior) margin are found many yellow pigment granules, as has been described by Hoffmann ('86). These granules are found within the cell, sometimes apparently in the nucleus (fig. 32). As Hoffmann stated, they are first seen in the bucco-pharyngeal membrane, but later occur also in the wall of the stalk. Müller ('71) had described them in the stalk in Acanthias embryos of 30 mm. length.

In 28 mm. embryos the lumen in the stalk is small. The wall consists of a double layer of epithelial cells, the outer of which



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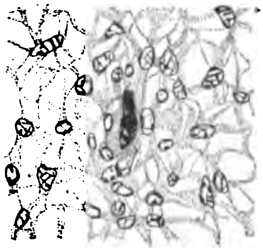

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Fig. 42 Transverse section of the hypophyseal stalk of a 37 mm. embryo. (H.E.C. Series 363). × 350.

Fig. 43 Transverse section of the hypophyseal stalk of a 41.5 mm. embryo. (H.E.C. Series 369). × 350.

is low columnar in type (fig. 40); the inner is irregular and the nuclei are oval in outline but not regularly placed. Figure 40 is of a section through approximately the middle of the stalk. The lumen and stalk are larger where it connects with the hypophysis and with the mouth, both ends being funnel-shaped and joined by a narrower middle part. The connective tissue around the stalk is mesenchymal in character. Immediately around the stalk the cells are concentrically arranged. This character is more pronounced in later stages.

In a 33 mm. embryo the two ends of the stalk are funnel-shaped, as before, and contain a lumen, while the middle part of the stalk is made up of a mass of cells in which no lumen is present (fig. 41). The outer layer of cells, though still definite at the ends, is no longer so in the middle part. The nuclei are more spherical in shape and contain a denser chromatin network. The arrangement of the mesenchymal cells around the stalk is concentric.

In a 37 mm. embryo the stalk is greatly reduced in size. The nuclei are massed in the center and are surrounded by a densely staining cytoplasm (fig. 42). Some pigment is scattered throughout the stalk, as in all of the specimens. The concentric arrangement of connective tissue cells is more marked.

In some embryos 40 mm. in length no remnant of the stalk is found. In a 41.5 mm. embryo a small elongated densely staining stalk is present (fig. 43). What apparently is the remains of the nuclear mass is surrounded by a narrow densely-staining cytoplasmic rim. The chromatin network has disappeared, but larger masses of densely staining chromatin are to be seen. The concentric arrangement of the connective tissue cells is apparent, but not so marked as in the younger stages. In another specimen, 40 mm. in length, a strand of connective tissue, extending from a funnel-shaped mass of epithelial cells—continuous with the epithelial lining of the mouth—to the base of the hypophysis, indicates the position of the degenerated stalk (fig. 8). The hypophyseal attachment of the stalk is anterior to the groove connecting the inferior lobes. Soon after this time the cartilages at the base of the brain become continuous across the mid line.

I wish to thank Dr. R. E. Scammon for his many helpful suggestions throughout this work. Thanks are also due to Dr. R. J. Terry for his kindly interest during its completion.

SUMMARY

1. The terms 'anterior lobe,' 'inferior lobes' and 'superior lobe' have been used for the several parts of the hypophysis of Acanthias.

2. Rathke's pouch forms the posterior part of the anterior lobe. The later evagination of the ectoderm, anterior to this, forms the middle portion and the anterior extremity of the anterior lobe.

3. The inferior lobes develop from the lateral sides of the posterior extremity of the anterior lobe, i.e., from the lateral sides of Rathke's pouch.

4. The superior lobe develops from the caudal (superior) end of the hypophyseal anlage.

5. In the course of development the hypophysis shifts in position about 145 degrees, so that the upper wall becomes the floor and the ventral (anterior) surface the roof.

6. There is glandular growth from the roof of the superior lobe and the inferior lobes, as has been described by Sterzi and others, and from the floor of only the anterior and posterior extremity of the anterior lobe in all adults.

7. The cells of both anterior and inferior lobes are acidophilic in character.

8. The cell columns of the superior lobe are solid as Sterzi described them.

9. Frequently the anterior and inferior lobes stain more densely than does the superior lobe. In these cases, it is the nuclei which take the darker stain. In general the anterior and inferior lobes may be considered the chromophilic ones.

10. Spaces containing some colloid-like secretion are present in the superior lobe. A similar secretion is present in the lumina of the tubules of the anterior and inferior lobes and in the large main lumen.

ADDENDUM

After the completion of the present work a paper by M. W. Woerdeman: "Vergleichende Ontogenie der Hypophysis" appeared (Arch. f. mikr. Anat., Bd. 86). This investigator figured Rathke's pouch in an 8 mm. Torpedo embryo. In somewhat older embryos (12–15 mm.) the region where Rathke's pouch opens into the mouth evaginates and in still later embryos a region anterior to this is constricted from the mouth. The hypophysis then consists of a small Rathke's pouch somewhat constricted from an anterior (ventral) 'Mittelraum' and anterior to the latter, the 'Vorraum.' The middle division, in 20 mm. embryos, divides by a circular constriction into a dorsal and a ventral part. In this way the ventral sacs are formed. The hypophyseal stalk now opens into the ventral sacs, in which observation Woerdeman agrees with that of Gentes and Stendell. According to Woerdemann's comparison of the parts of the hypophysis with those described by Stendell, Rathke's pouch is homologous with the superior lobe (table 1, p. 400) and the 'Mittelraum' and 'Vorraum' are homologous with the anterior lobe. The ventral sacs and lateral lobuli which he described are probably homologous with the inferior lobes. In Squalus I have described Rathke's pouch, or the early anlage of the hypophysis as giving rise to the caudal extremity of the anterior lobe. A later evagination ventral to this gives rise to the middle portion and anterior extremity of the anterior lobe. The secondary evagination is early recognized, as the epithelium here is thickened (page 408). The opening from the mouth to the early hypophyseal anlage, or Rathke's pouch, secondarily comes to open in the later evagination (page 410) of which there is only one in Squalus. The inferior lobes and the superior obe develop from the early hypophyseal anlage in Squalus, as has been described (pp. 410–11). The hypophyseal stalk is not constricted from the anterior lobe with the developing ventral lobes but remains connected with the caudal wall of the middle portion of the anterior lobe until it disappears.

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February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

49. The development of the gall bladder and bile ducts in amblystoma.

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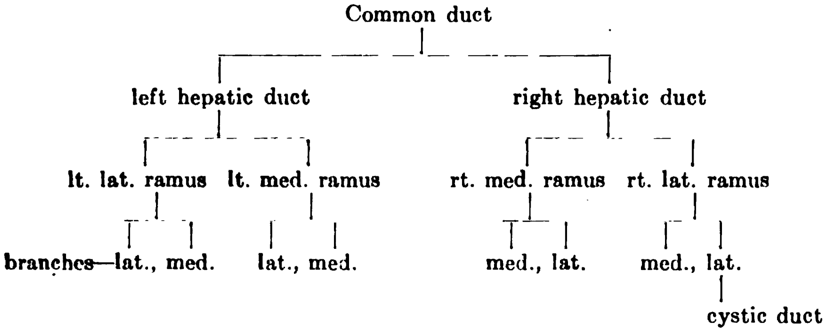
Models of embryos 4.5 mm. long show that the liver anlage is a ventral, somewhat caudal projection of the gut lumen caudal to the heart. That this does not correspond to the caudal hepatic duct described in chick is shown by later stages. In models of embryos about 7 mm. long we see this early ventral outpouching has turned cranialward. In the ventral wall where the primitive common duct joins the gut lumen a median depression shows the earliest anlage of the gall bladder. In stages of about 9 mm. length the folds have become numerous and are the anlage of radicles of the main hepatic ducts. That they are not entirely formed by the tunneling in of blood vessels as has been described by Shore, is shown by the fact that furrows are found in which no blood vessels are present. The early anterior, and laterally extending duct shows beginning constriction and elongation into right and left hepatic ducts. A little later the gall bladder has shifted to the right and is no longer widest transversely. The cystic duct also has shifted to the right, now being attached to the ventral side of the right hepatic duct. The shifting of the gall bladder is accompanied by a shifting of the entire caudal part of the liver due to the sinistral and ventral growth of the stomach and duodenum.

From graphic reconstructions of late embryos we see an arrangement of the biliary apparatus as shown in the following outline:



Occasionally the left medial and right medial rami join to form one duct which subdivides as a single stem. This is a secondary union and persists in the adult. Also in some cases the cystic duct was found to be one subdivision further removed, that is, it was attached to a radicle of the lateral branch of the right hepatic ramus.

In embryos between 10 and 12 mm. in length, division and growth of the early duct into right and left hepatic ducts and of these into rami takes place. A graphic reconstruction of a 13 mm. stage shows that this has taken place. The gall bladder here is now longest postero-anteriorly and the short cystic duct extends upward and to the left. A model of a 14 mm. embryo shows that the gall bladder has shifted more to the right and that its duct which is attached to the extreme anterior end extends somewhat upward and to the left.

A model of the biliary apparatus of a slightly older embryo shows greater development of the duct system. The gall bladder is longer anteroposteriorly, its duct is attached more caudally and is almost horizontal. In a 15 mm. embryo there is a short common duct, but the right and left hepatic ducts are longer. The right lateral shifting here is more marked. The cystic duct extends horizontally to the left. This stage marks the extreme caudal attachment of the cystic duct to the gall bladder.

At the 20 mm. stage the lateralward shifting is very marked. The right hepatic duct is now somewhat dorsal to the left. Here the right and left medial rami have united to form one duct. The gall bladder has increased very materially in size, particularly in its caudo-cranial diameter. The cystic duct has shifted toward the anterior end and now projects to the left and downward. The extreme of the lateralward shifting has been reached in a 35 mm. embryo. The right hepatic duct here is quite dorsal in position in relation to the left. Its lateral radicles are also more dorsal than its medial. The left lateral ramus has turned caudalward to supply hepatic radicles to the further caudal extending left lobe of the liver. The gall bladder has increased in its dorso-ventral diameter. Its duct is attached at the anterior end and extends toward the left and downward as before. In a 45 mm. embryo and larger ones the right and left hepatic ducts are again more nearly in a horizontal plane. The cystic duct is attached to the anterior medial end of the gall bladder.

Summary. (1) The ductus choledochus develops as the early anterior directed duct from the gut. (2) The right and left hepatic ducts develop as divisions of the ductus choledochus and by growth and division form the hepatic rami and branches. (3) The gall bladder begins as a median ventral outpouching of the early anterior forming liver anlage. It is first widest laterally and finally obliquely caudo-cranially. (4) There is an early right lateral shifting of the biliary apparatus. Along with this there is a constant shifting direction of the cystic duct in keeping with the dorsalward migration of the gall bladder. (5) The cystic duct is early closed off with the right hepatic and finally is attached to the lateral branch of the right lateral ramus.

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ON THE VASCULARIZATION OF THE SPINAL CORD OF THE PIG

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FIVE FIGURES

HISTORICAL

Until 1904 little work had been done upon the development of the blood-vessels of the spinal cord, except that of His ('86) who undertook to follow the growth of these vessels in human embryos. The observations of this author have been largely disputed by Sterzi ('04) and Evans ('09), the two writers who have done most of the work in this field.

By far the most comprehensive publication upon the development of the vessels of the spinal cord is that of Sterzi ('04). In this he discusses the development of the vessels in the five higher classes of vertebrates. As a type of the Mammalia he uses the sheep. He brings out the following points:

The blood-vessels first approach the cord at the ventro-lateral border and spread over the ventral surface, then over the lateral, and finally over the dorsal surface. Each vertebro-medullary artery as it approaches the cord divides into a ventral and a dorsal ramus, the ventral and dorsal radical arteries. The ventral radicals from either side halt at the lateral edges of the floor-plate, and each divides into a cranial and a caudal branch. These anastomose with those of adjacent segments and form two longitudinal arteries on the ventral surface of the cord, the "tractus arteriosus primitivus." Later they send out medial rami and through these become connected. Still later, alternate parts of the two tracts degenerate while other parts continue to develop. These enlarged segments are joined together through their medial rami and form a single ventral artery which Sterzi terms the tractus arteriosus ventralis, and which is the anterior spinal artery

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of most authors. From the primitive tract, dorsal rami enter the substance of the cord. Each dorsal ramus forms a loop and gives rise to a vein, which courses ventrally and enters the primitive sulcus. Later other vessels extend into the cord from the lateral, and still later from the dorsal surface.

The dorsal radical arteries, where they divide, form many small longitudinal capillaries just ventral to the points of emergence of the dorsal nerve roots. From these capillaries there is formed later a longitudinal artery on either side of the cord, in this plane (tractus arteriosus lateralis). The vessels entering the cord are first solid and later become hollow.

Evans ('09) shows by a series of injected pigs the early development of anterior spinal artery In his figures the mid-ventral surface of the cord is shown to be free from vessels until the embryos are 8.5 mm. in length, and the mid-posterior surface until after the pigs are between 8 and 10.5 mm. in length. He does not take up the later stages.

MATERIAL AND METHODS

For a study of this nature, injected embryos are indispensable, and they are best injected while living, with warm india ink diluted one-half with weak ammonia water. It is preferable to inject through the umbilical artery rather than through the umbilical vein, because the arteries are less readily ruptured and because the route between them and the vessels of the spinal cord is much more direct.

Embryos used for thin serial sections are better if they are congested instead of being injected. This congestion is accomplished as follows: The umbilical cord is tied while the embryo is yet living, thus causing an increase in the blood pressure in the aorta. One of the most direct outlets for this increased pressure is the system of segmental arteries, and through these the bloodvessels in and around the spinal cord soon become engorged. When this condition is reached, as evidenced by the increased redness of the dorsal region of the embryo, the live embryo is dropped into a fixing fluid which penetrates rapidly so that the capillaries are fixed before they collapse. Bouin's picro-formo-acetic mixture serves this purpose very well. Embryos treated in this manner show the smaller vessels much more plainly than those fixed in the usual way.

Small injected embryos which have been cleared in oil may be dissected under the binocular microscope and all the external vessels of the cord demonstrated, or they may be sectioned in celloidin to show the internal vessels.

From pigs larger than 25 or 30 mm. the cord with its membranes may be dissected out and embedded in celloidin and cleared, or for temporary preparations may be cleared directly.

Serial sections of the cleared embryo or spinal cord can be kept permanently between two pieces of paper soaked in oil, or can be transferred to slides and mounted in damar gum. To make slide preparations, the sections are cut in oil and placed on a piece of thin paper in the same order they are to have on the slide. Another piece of oiled paper is laid down upon them and the whole inverted. The first paper is now peeled off and the other paper holding the sections is inverted upon the slide. This paper is then peeled off, leaving the sections on the slide in their proper order. They may then be washed carefully with xylol, and covered.

There are several advantages in a study of this kind, in section ing celloidin-embedded embryos free-hand in oil. The cord can be turned so that sections may be cut through any plane. Sectioning is done more rapidly than with a microtome, and much time is saved. It is easy to make transverse, sagittal and frontal sections from any region of the same cord. The block can be examined with a lens, during the sectioning, and any particular vessel or vessels included in a section. Also, sections may be made of different shapes.

BLOOD-VESSELS OF THE NEAR FULL-TERM FETUS

In order to determine as near as possible the arrangement of the blood-vessels of the spinal cord in the adult condition, a number of fetal pigs, near full-term, were injected and their spinal cords dissected out for study. Although these showed some variation in the blood-vessels, as is to be expected, a general plan was to be observed. The following description is of the typical condition found in the blood-vessels in and around the spinal cord of pigs of about 240 mm. in length:

The terminology used by Kadyi ('89) for the blood-vessels of the adult human cord will be referred to frequently.

On the surface of the cord at this stage are four main longitudinal arterial systems which are located: one, median on the ventral surface; one, on each dorso-lateral surface; and one median on the dorsal surface.

The vertebro-medullary branches of the dorsal segmental arteries approach the cord laterally and each divides into a dorsal and a ventral branch termed the posterior and ventral radical arteries respectively (figs. 1 and 2). The latter reach the cord along the cranial surface of the spinal nerves and ganglia, and, on the cord, run cranially. They may be equally distributed to the two sides of the cord and to the different regions of it, or some regions may have more than others. They average eighteen in number. The ventral and dorsal radical arteries have lost their connection with the vertebro-medullary artery in places and extend between the artery on the ventral surface of the cord and one of those on the dorso-lateral surfaces.

Soon after reaching the cord each ventral radical artery branches, giving off one ramus which courses cranially, and another which extends caudally. These divisions anastomose with those of neighboring ventral radicals, on the same or opposite side

Fig. 1[1] Ventral surface of the middle thoracic region of the spinal cord of a fetal pig, 240 mm. in length. *A.S.A.*, anterior spinal artery; *A.R.V.*, ventral radical artery; *T.A.P.*, accessory anterior spinal artery (remains of primitive arterial tract); *V.R.V.*, ventral radical vein; *V.S.A.*, anterior spinal vein; *C.P.*, capillary plexus. × 6.

Fig. 2 Dorsal surface of the same part of the cord shown in figure 1. *A.M.D.*, median dorsal artery; *A.P.L.*, dorso-lateral artery; *A.R.D.*, dorsal radical artery; *V.M.D.*, median dorsal vein; *V.P.L.*, dorso-lateral vein and plexus; *V.R.D.*, dorsal radical vein; *V.L.*, dorso-lateral venous plexus. × 10.

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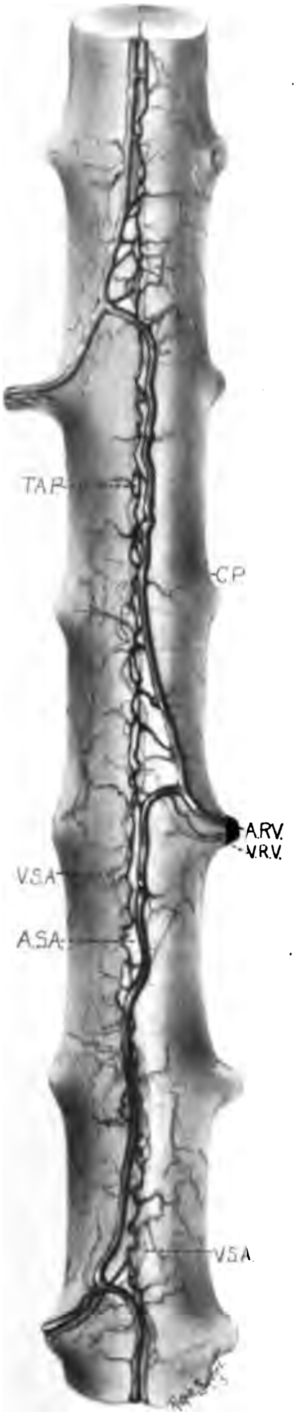
[1] The figures in this paper, except numbers 3 and 4, were drawn with a camera lucida. The size of the embryos is the greatest length, as measured in 75 per cent alcohol.



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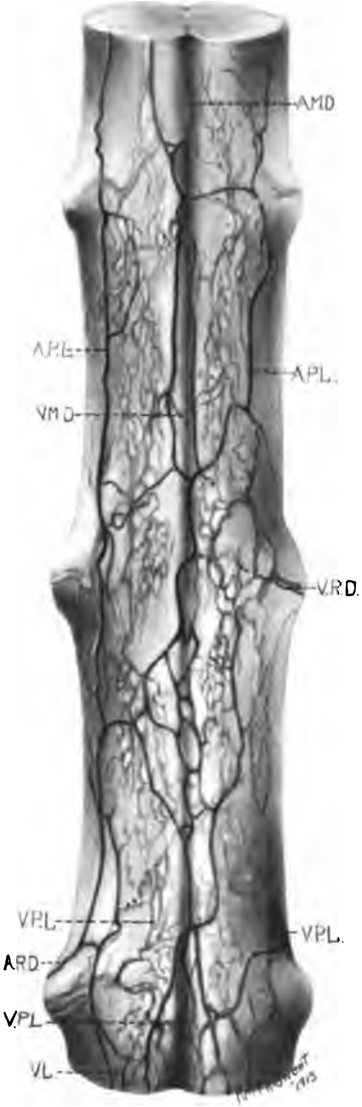

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of the cord, and form in this way the anterior spinal artery, which lies in or near the median ventral line. Occasionally a radical artery, instead of dividing, goes across the cord and joins the cranial or caudal ramus of the one on the opposite side. The anterior spinal artery has a winding course, bending laterally to meet the vessels which form it, and making many smaller irregular bends to one side or the other. In some places it may lie to one side of the mid-line for several segments and where this occurs there are found numerous longitudinal rami of the anterior spinal artery, or the cranial and caudal divisions of the ventral radicals. These may be called accessory anterior spinal arteries, and are sometimes present even where the anterior spinal artery lies in the mid-line. Here they are located between this vessel and cord, at the lip of the ventral median fissure. They are the remnants of the "tractus arteriosus primitivus" of Sterzi.

The dorsal radicular rami of the vertebro-medullary arteries are much more numerous than the ventral ones. They course dorsally along the cord and in a slightly cranial direction, to a plane just ventral to the emergence of the dorsal roots of the spinal nerves, where they divide into two rami, one extending cranially and one caudally. Each of these rami anastomoses with the one of the adjacent segment, and thus there is formed on either side an irregular longitudinal vessel, the dorso-lateral artery (fig. 2; tractus arteriosus postero-lateralis of Kadyi). From this artery recurrent rami supply the dorsal nerve roots and spinal ganglia, and the lateral surface of the cord. Other rami, two or three in each segment, and much larger than the above, run dorsally and by longitudinal anastomoses with each other, and with similar rami from the opposite side, form an artery in the mid-dorsal line which may be termed the median dorsal artery.

Very small rami from the dorso-lateral arteries run ventrally along the cord and unite with others from the anterior spinal artery, forming a plexus on the ventral and lateral sides. These ventral rami of the dorso-laterals anastomose freely in a longitudinal direction and form one or more small longitudinal arteries between the ventral and dorsal nerve roots in some parts of the cord. These are the tracti arteriosi laterales, and ventro-laterales, of Kadyi. Still other small rami from the dorso-lateral and median dorsal arteries form a capillary plexus on the dorsal and dorso-lateral surfaces of the cord. In a few places along the cord the dorso-lateral arteries are double, one division lying dorsal to the dorsal nerve roots, and corresponding perhaps to the "tractus arteriosus posterior" of Kadyi.

The median dorsal artery is a very irregular longitudinal vessel formed by the dorsal rami of the dorso-lateral arteries, as described above. In places it is double or may show a longitudinal capillary arrangement. Many of its lateral rami anastomose longitudinally forming small arteries parallel with the median dorsal artery (fig. 2). This is also true of the dorsal rami of the dorso-laterals. By the anastomoses of the rami of the various arteries just described, the entire cord is surrounded by an arterial vascular system, and from all parts of this network smaller arteries penetrate its substance.

The veins of the spinal cord are in three principal longitudinal systems, and other smaller ones. Of the three, two are dorsal and one ventral. All three show evidence of their capillary origin. The anterior spinal vein is the smallest of the three (fig. 1). It lies between the cord and the arteries, in the median ventral line. It is larger than the accessory anterior spinal arteries, but never attains the size of the anterior spinal artery proper. It is very irregular and in some regions is entirely replaced by a narrow network of capillaries.

On either side of the median ventral sulcus, the cord is covered with large venules, some of which lie between the cord and the arteries, and some of which are external to the latter. They are often two or three times as large as the arterioles to which they correspond. They anastomose freely with the anterior spinal vein and empty laterally into the ventral radical veins which are in close relation with the ventral nerve roots and ventral radical arteries, but which are much more numerous than the latter, one being present on nearly every nerve root. They drain blood also from the lateral surface of the cord. Their ventral and dorsal rami often form short, small, longitudinal veins by anastomoses, some of which in other animals have been named, antero-lateral, etc. The blood from the anterior spinal veins and venous capillaries of the general ventral surface form, in places, transverse channels which are perhaps large enough to be called veins.

On either side of the dorsal surface of the cord there extends longitudinally a large irregular vein, the dorso-lateral, about half way between the artery of the same name, and the median dorsal artery. Some parts of these vessels and their rami, like the ventral venous capillaries, lie external to the arteries and some internal to them. They are the largest vessels on the cord with the exception of the anterior spinal artery (compare figs. 1 and 2). Dorsally these veins are united through large capillaries, and blood leaving the cord in the median line may flow either to the right or left. Half way between two consecutive nerve roots the dorso-lateral veins usually break up into many divisions so that each may be seen to drain blood from adjacent halves of two segments. Laterally they empty through one or more divisions into the large dorsal radical veins, one of which lies upon each dorsal nerve root (fig. 2).

A fourth longitudinal venous system, smaller than the three described, lies in the median dorsal sulcus. It resembles the dorso-lateral veins except that it is more irregular, and in places it may be entirely lacking. Its lateral rami empty in the dorsal venous capillary plexus or directly through larger vessels into the dorso-lateral veins. It may be termed median dorsal venous system.

Some of the venous capillaries of the lateral surface drain into the dorso radical veins, some into the dorso-lateral veins, and some into ventro radical veins, and all these vessels together with the anastomoses of the veins on the ventral and dorsal surfaces already mentioned above, completely surround the cord with a venous system, corresponding to the system described for the arteries.

Of the arteries entering the cord, the largest are those in the ventral fissure, the ventral central arteries, which form two nearly parallel rows, but which are not paired. They arise from the anterior spinal arteries, or the accessory anterior spinal arteries. They show evidence of the capillary origin in longitudinal anastomoses found between vessels of the same side. These anastomoses are numerous in the fissure, particularly near the vessels from which the ventral central arteries arise.

The ventral central arteries vary considerably in size, some being as large as the vessels they arise from and others much smaller (fig. 3). The course of the smaller vessels is usually more irregular than that of the larger. They pierce the substance of the cord at different levels, some entering near their origin and others extending some distance into the fissure. Their general course is dorso-lateral, but those entering near the mouth of the fissure may bend very sharply to the side and enter the ventral horn of the gray substance. The others course more dorsally nearly to the level of the central canal where they make a decided lateral bend, and divide into two or more rami, although sometimes they give off rami more ventrally than this (figs. 3 and 4). The principal divisions of these arteries extend in a longitudinal plane, and anastomose with similar rami of adjacent vessels. They also give off smaller arteries and capillaries which ramify through the gray matter in all directions, helping to form a dense plexus. The longitudinal arteries tend to form loops after they have coursed in one direction for a short distance, as they do in young embryos (fig. 5). One artery may form several such loops, producing as many longitudinal vessels, each succeeding vessel lying dorsal or lateral to the last, and of a lesser caliber. These smaller longitudinal vessels anastomose with each other ventro-dorsally and laterally by rami which usually leave them at right angles, and also anastomose with rami from vessels other than the ventral central arteries, as will be described later.

Besides the rami of the central arteries just described, other rami extend farther laterally into the gray substance before branching. Some of these, instead of forming longitudinal vessels, form small irregular ones which ramify through the gray matter in all directions, anastomosing with similar vessels from other arteries in this region and forming a dense capillary plexus in the ventral and dorsal horns.

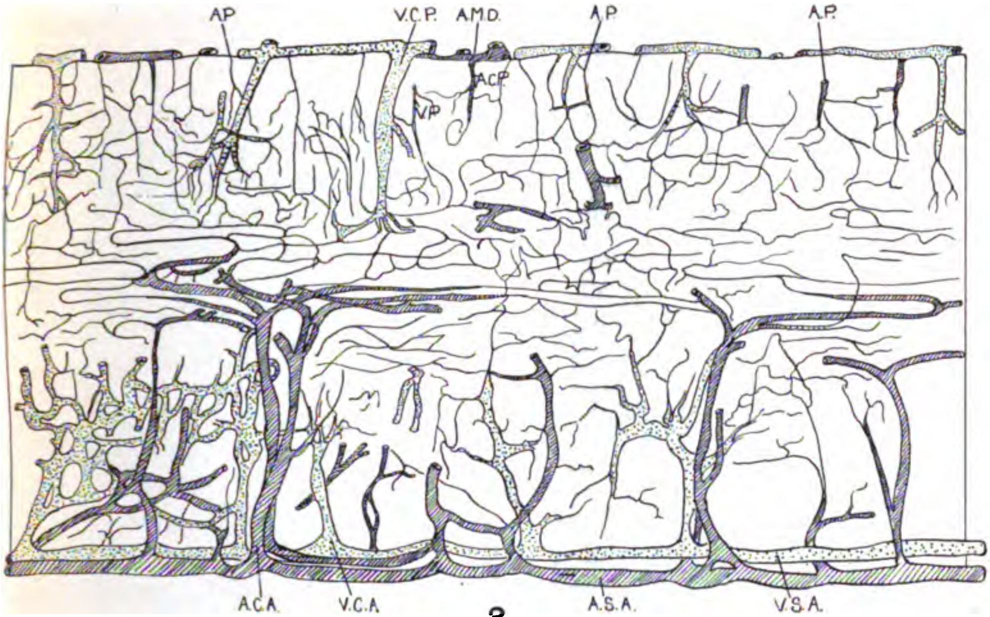
Other arteries, smaller than the ventral central arteries, enter the cord from the dorsal median sulcus and course ventrally and laterally to the dorsal horns of the gray substance. Here they form still smaller vessels resembling to some extent those formed by the ventral central arteries, but most of their rami are short and do not extend longitudinally. These may be called the dorsal central arteries, but they are more similar to the peripheral arteries from other surfaces of the cord, than to the ventral centrals, and perhaps should be called dorsal peripheral arteries. They give off many small lateral rami in the white substance and in the outer part of the gray substance.

In addition to these vessels, other small arteries enter the cord from all sides, from the arteries and arterioles which surround it. These are the peripheral arteries referred to above. They are very numerous and in a single thick cross section as many as fifty or sixty of them may be counted. They give off short rami in the white layer of the cord and extend into the gray layer. These rami branch and anastomose and form a loose capillary network. The vessels entering the gray substance enter into the longitudinal plexus already described and give off lateral rami which branch freely and anastomose.

The longitudinal vessels which arise from the ventral central arteries are quite large, but the other vessels formed from these trunks, and those formed from the dorsal central and peripheral arteries, are much smaller. A very thick section presents a picture of an inner core of longitudinal vessels with other vessels extending into it at right angles from all points on the periphery of the cord (figs. 3 and 4).

Fig. 3 Sagittal section from the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.C.A.*, ventral central artery; *A.P.*, peripheral artery; *A.C.P.*, dorsal central artery; *V.S.A.*, anterior spinal vein; *V.C.A.*, ventral central vein; *V.C.P.*, dorsal central vein; *V.P.*, peripheral vein. × 35.

Fig. 4 Transverse section through the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.P.*, peripheral artery; *A.*, artery; *A.C.A.*, ventral central artery; *S.A.S.A.*, accessory anterior spinal artery; *A.P.L.*, dorso-lateral artery; *A.M.D.*, median dorsal artery; *A.S.V.*, anterior spinal vein; *V.*, vein; *V.P.*, peripheral vein; *V.P.L.*, dorso-lateral vein; *V.M.D.*, median dorsal vein. × 35.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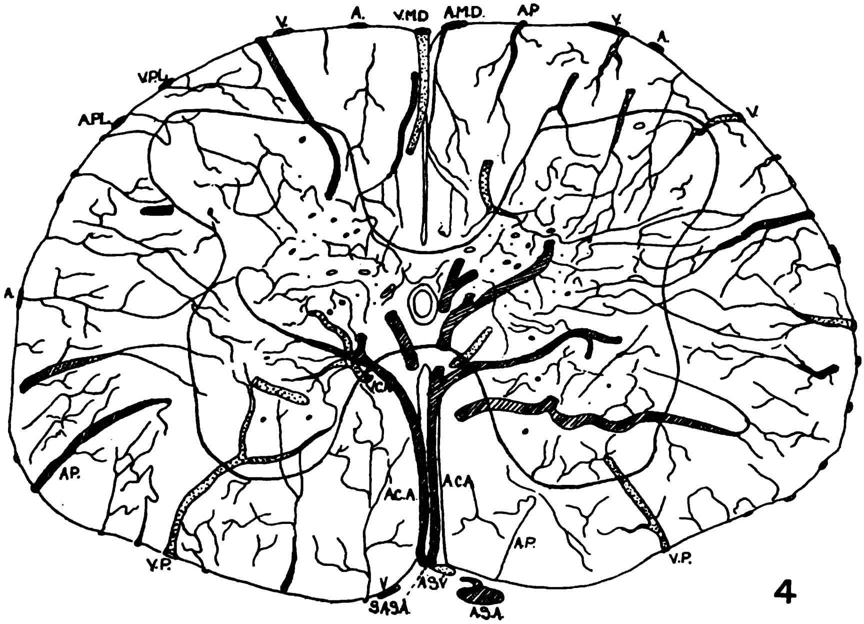
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4

DEVELOPMENT OF THE BLOOD-VESSELS

The early development of the anterior spinal artery has been described by Evans ('09) and Sterzi ('04).

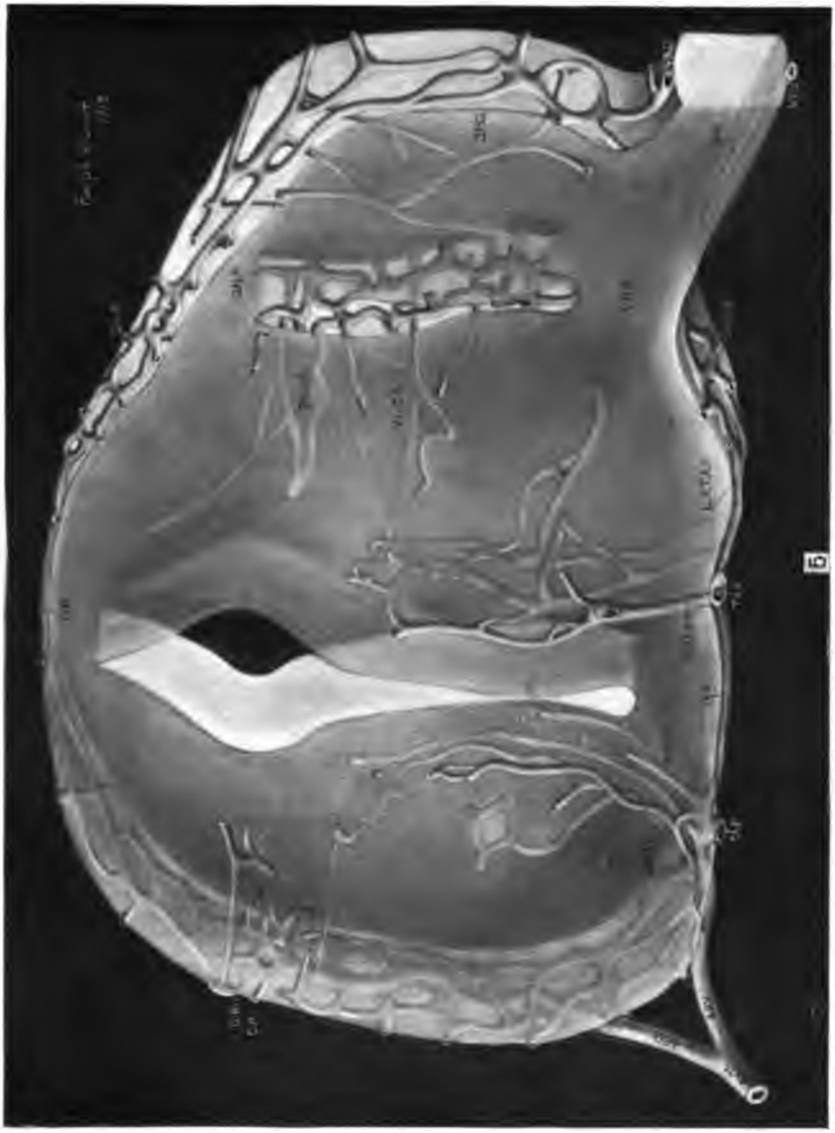
Some pig embryos of 12 mm. show a fairly well developed anterior spinal artery, while in others of 14 or 15 mm. it is just beginning to form. Although, after this vessel is once formed, it does not undergo marked changes, there is some modification. For example, the ventral radical arteries meet it at right angles or nearly so, until the embryo is 40 or 45 mm. in length. Thereafter, the growth of the cord and the fixed position of the radicals seem to cause the artery to be pulled laterally by the radicals, and a gradually decreasing angle is formed at the points where the radicals meet it (fig. 1).

As the embryo grows, the number of radical arteries continues to decrease even after the anterior spinal artery is well formed. This seems to be true until the embryo reaches the length of about 100 mm.

Some of the arterial capillaries on the ventral surface of the spinal cord are continuous with the anterior spinal artery directly, or indirectly through remains of the tractus arteriosus primitivus, and others are continuous with the capillaries of the lateral surfaces of the cord.

A dorso-lateral artery is formed in the capillary plexus on each of the lateral surfaces of the cord, just ventral to the point of emergence of the dorsal nerve roots. The dorsal radical arteries branch in this region and give off dorsal and lateral rami, which are continuous with the lateral capillaries just mentioned. A very irregular longitudinal vessel develops where certain of these

Fig. 5 Transverse section through the mid thoracic region of the spinal cord of an 11 mm. pig embryo. *T.A.P.*, primitive arterial tract; *R.D.T.A.P.*, *R.L.T.A.P.*, *R.M.*, dorsal, lateral, and medial rami of the primitive arterial tract; *A.V.M.*, vertebro-medullary artery; *A.R.V.*, *A.R.D.*, ventral and dorsal radical arteries; *C.R.*, *Cr.R.*, *D.R.*, *V.R.*, caudal, cranial, dorsal and ventral rami of the dorsal radical artery; *D.P.*, *D.L.P.*, dorsal and dorso-lateral capillary plexuses; *D.L.C.G.*, *V.L.C.G.*, dorso-lateral and ventro-lateral groups of peripheral capillaries; *V.L.V.P.*, ventro-lateral venous plexus; *V.R.V.*, *V.R.D.*, ventral and dorsal radical veins; *S.P.G.*, spinal ganglion; *V.N.R.*, *D.N.R.*, ventral and dorsal nerve roots; *S.N.*, spinal nerve. × 200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capillaries increase in size, perhaps on account of the increased pressure from the dorsal radical arteries. This longitudinal vessel is indicated in embryos of 12 mm. and is quite strongly developed in embryos of 15 to 18 mm. In these stages it seems to dip ventrally to meet the approaching radicals, as pointed out by Sterzi for the sheep ('04). As the embryo grows, this dorso-lateral artery becomes more and more regular. It is still somewhat irregular in embryos of 60 mm. but quite regular in those of 75 mm. The dorso-lateral artery never attains the size of the anterior spinal nor is it ever so regular in its course. In places it may develop as two or more vessels, but these are always smaller than the single artery. The dorso-lateral arteries are each continuous with the capillaries of half the cord in the early stages, but as the cord increases in size they supply directly only the dorso-lateral surface.

The capillary network on the lateral surface of the cord is at first continuous with that extending through the mesenchyma of this region as far, laterally and dorsally, as the myotomes and body wall respectively. In later stages when the membranes of the cord begin to develop, the connections between the vessels of the cord and those in the mesenchyma around it are lost.

The median dorsal artery is the last of all the vessels on the cord to develop. In pigs of 30 mm. it is still very irregular and indefinite, and is entirely lacking in places, although the vessels which go to form it, the dorsal rami of the dorso-lateral arteries, may be seen in embryos of 20 mm. In pigs of 45 mm. it is quite definite, lying in or near the mid line of the dorsal surface, as described above for the 240 mm. embryo It never becomes very regular, and in pigs of 100 mm. it resembles the condition seen in the pig of 240 mm. It is continuous with the arterial capillaries of the dorsal surface of the cord and with the dorso-lateral arteries.

In addition to these main arterial trunks there develop on various parts of the cord, especially on the lateral surfaces, short longitudinal arteries. These are never large or regular. They have been described in connection with adult human cord under the terms "tractus arteriosus; ventro-lateralis, posterioris, and lateralis" (Kadyi). Of these, the "tractus arteriosus posterior" is the most prominent and corresponds to the description in this paper of parts of the dorso-lateral artery, where it sometimes has two divisions, one of which runs dorsal to the dorsal nerve roots and the other ventral to them. The dorsal divisions are evidently the same as this 'tractus.'

The veins on the cord develop in much the same way as do the arteries. The ventro-lateral surface of the cord in very young embryos is covered with capillaries, and these are continuous laterally with the capillaries in the mesenchyma round the neural tube. Medially they become continuous with the lateral rami of the primitive arterial tract. When this tract becomes separated from the cord by the ingrowth of mesenchyma, these capillaries send medial outgrowths between the tract and the cord, as seen in embryos of 12 to 15 mm. Dorsally they grow along the cord and spread over the dorso-lateral surface (pigs of 6.2 mm.) and later over the dorsal surface (pigs of 7.5 mm.). Laterally they spread over the ganglia.

From the ventral surface, the blood draining away through the capillaries soon establishes segmental vessels, the ventral radical veins, which course laterally along the nerve roots. Each radical vein on one side drains adjacent halves of two segments. These receive blood from the capillaries of the ventral, lateral, and ventro-lateral surfaces. Lying in the ventral median fissure in young embryos, small longitudinal veins may be seen in different regions of the cord, and in embryos of 25 to 30 mm. a fairly definite longitudinal vessel may be found here. This vessel in still older embryos becomes a more definite trunk and may be called the anterior spinal vein. It never attains the size of the anterior spinal artery. Laterally it drains into the ventral radical veins.

Some of the ventral and lateral capillaries of the younger embryos, early become differentiated into veins. This is especially true of the dorsal vessels. From these, some of the blood drains laterally out through vessels in the mesenchyma to the myotomes. A pig of 6.2 mm. shows three planes in which this occurs, one on a level with the dorsal surface, one just above the level of the ventral surface, and one about half way between the other two. At the myotomes the blood drains ventrally into the intersegmental veins. Some of the capillaries of the lowest of these three planes, which drain the blood from the lateral surface of the cord and from the ganglia, soon become large and are called the vertebro-medullary veins, one pair of which is formed for each segment. In older embryos they course along the spinal nerves with the vertebro-medullary arteries. They receive the blood from the ventral and dorsal radical veins. The former have been described. The latter develop along the sides of the ganglia in the capillaries already mentioned. At first they carry only a part of the blood from the dorsal surface of the cord, but later (pigs of 25 mm.) they carry practically all of it. They are more numerous than the corresponding ventral radicals, and are found in every segment.

The venous capillaries of the dorsal-lateral surface on either side draining toward the nerve roots early establish longitudinal veins. These are only about half as long as a segment of the cord. Figure 5 of an 11 mm. pig, shows an indifferent plexus on this surface, but in 15 to 17 mm. embryos, fairly definite vessels may be seen. These become more and more regular as the animal develops, and as embryos of 50 to 60 mm. show, they form a venous system on either side of the cord just dorsal to the dorsal nerve roots, much like that described for the 240 mm. stage. These systems constitute the dorso-lateral veins (fig. 2).

The first blood vessels entering the cord grow in as capillaries from the ventral surface. Sterzi ('04) reports vessels in the cord of a sheep of 5.5 mm., but they were not apparent in the cord of pig embryos of less than 7.5 mm. These vessels are the dorsal rami of the primitive arterial tracts, of the lateral rami of these tracts, and of the other capillaries near the median line. They are the first indications of the central arteries and veins. They form two nearly parallel rows, one on either side of the ependymal layer, or some of them may lie in this layer. They grow dorsally about half way to the dorsal surface of the cord. They exhibit numerous longitudinal anastomoses and form a plexus along the ateral side of the ependymal layer in each half of the cord. These are true capillaries at first, but soon differentiate into arteries and veins.

Those coming directly from the primitive arterial tracts all become arteries, while those coming from the vessels lateral to the tract may become either veins or arteries.

In embryos of 9.5 mm. another group of capillaries may be seen to have entered the cord. These come from the lateral surface, extending medially nearly to the central canal. Later they anastomose ventro-dorsally and longitudinally, among themselves and with the vascular sprouts from the ventral surface.

The vessels in the cord of a pig of 11 mm. present the following characteristics, as shown in figure 5. Rami from the primitive arterial tract may anastomose with those from the ventral capillaries. Neighboring vessels of the same kind anastomose freely and give off lateral rami into the anlagen of the ventral horns of gray substance. These rami branch and anastomose with each other and form loops which anastomose with the central vessels from which they arise, or with neighboring vessels. In a plane just above the anlagen of the ventral horns each of the central vessels ends blindly, or divides into a caudal and a cranial ramus, which anastomose with adjacent similar rami and form irregular longitudinal vessels. By other anastomoses among the central vessels, a longitudinal plexus is formed, which covers very completely the lower half of the lateral side of the ependymal layer.

A comparison of figures 3 and 5 shows how closely the form and arrangement of these capillaries corresponds to that of the future central arteries and veins. Besides these main capillaries two smaller lateral groups are present at this stage. These may be called the ventro- and dorso-lateral groups, and later form peripheral arteries and veins. Both groups enter the cord from the capillaries on the lateral surface between the dorsal and ventral nerve roots. The ventro-lateral group enters at the level of the dorsal extremities of the central vessels, and courses medially and anastomoses with them. Occasionally the ventro-lateral group gives off rami which extend into the anlagen of the ventral horns. The capillaries of the dorso-lateral group are confined to the dorsal two-fifths of the cord, and although they anastomose with each other at this stage, they do not anastomose with the central or ventro-lateral capillaries. They course medially and dorsally along the ependyma, ending blindly or forming loops, but do not reach the dorsal surface.

As development proceeds, the lateral groups of capillaries shown in figure 5 spread dorsally and ventrally and capillaries enter the cord from the periphery. With the exception of the above-mentioned dorso-lateral group of capillaries, all the vessels entering the sides of the cord grow toward a common center, namely, an area on the lateral border of the ependyma about half way between the dorsal and ventral surfaces. The dorso-lateral group of capillaries which are shown in the same figure send rami toward this center after the embryo attains the length of 14 mm.

The vessels from the dorsal surface grow ventrally along the ependyma and unite with the dorsal rami of the primitive arterial tract. This union continues the plexus on the lower part of the ependyma dorsally so that the ependyma except below the floor-plate and above the roof-plate, is entirely surrounded by a capillary plexus. A thick transverse section of the cord of an embryo of 25 mm. shows this plexus with numerous vessels extending from it laterally at right angles. These lateral vessels are joined together by dorso-ventral rami. This picture is characteristic of the cord until the embryo reaches the length of 30 or 35 mm. when it is changed by other peripheral vessels meeting the ependymal plexus obliquely and by the branching of the vessels in the anlage of the gray substance.

By this time the central arteries from both the ventral surface (ventral central arteries) and from the dorsal surface (dorsal central or dorsal peripheral arteries) have become quite large, although the latter do not nearly equal the size of the former. The ventral central arteries have formed more longitudinal loops similar to those shown in figure 5. They are separated more and more from each other, owing to the growth of the cord, and as this separation continues the longitudinal vessels grow in length.

In embryos of 35 to 40 mm. in length the peripheral arteries from all sides together with the lateral rami of the central arteries have formed a dense plexus in the gray substance, although the white substance contains only the peripheral arteries running through it, and the short branching rami given off at right angles from them. By the time the embryo reaches a length of 50 mm. the capillaries in the white layer have much the same appearance as those of the full term fetus, except that in the latter they branch and anastomose more freely and the growth of the cord tends to separate both the peripheral vessels and the central vessels. Embryos of 75 to 100 mm. in length show the arteries in the cord quite as completely developed as in the 240 mm. embryo.

The posterior rami of the primitive arterial tract in the ventral part of the cord of embryos of 12 to 15 mm. are more numerous than the central arteries in the 240 mm. embryo which are formed from them.

The veins within the cord develop in the same planes as the arteries, and from the same plexus of capillaries that form the latter. They may be called the central and peripheral veins corresponding to the similarly named arteries. They are shown in figures 3 and 4 in a fully developed condition.

SUMMARY

The dorsal rami of the primitive arterial tract, and other rami from the capillaries in its immediate vicinity enter the cord, forming an undifferentiated capillary plexus (fig. 5) and this plexus later becomes differentiated into arteries and veins. It was not found, as stated by Sterzi for the sheep, that each dorsal ramus of the primitive arterial tract grows into the cord, and forms a loop, giving rise to a vein which grows back along the artery to the ventral surface.

The dorsal rami of the primitive arterial tract are more numerous than the ventral central arteries which develop from them.

Sterzi reports solid blood-vessels in the cord of sheep of 5.5 mm. and hollow ones in those of 6.6 mm. In pig embryos the blood-vessels within the cord seemed to appear first as hollow vessels. These are seen first in embryos of 7.5 mm. in length.

The "tracti arteriosi laterales" of Sterzi, are the dorso-lateral arteries of this and postero-lateral of other papers, and are the posterior spinal arteries of human descriptive anatomy. Evans shows these two tracts first united by medial anastomoses in a pig of 8.5 mm. in length, but many such anastomoses are to be found in embryos as small as 7.5 mm. in the cervical and thoracic regions, and one specimen of 6.2 mm. showed them in the cervical region.

The embryos described in this paper show the mid-ventral and mid-dorsal surfaces of the cord to be covered with blood-vessels at a somewhat earlier stage than has been described.

As reported by Sterzi ('04) and Evans ('09), blood-vessels first appear on the ventro-lateral surface of the cord, then on the ventral, then on the dorso-lateral, and finally on the dorsal surface.

The blood-vessels on the cord are continuous with those in the mesenchyma surrounding it until the membranes of the cord are formed.

It is generally stated in textbooks of human anatomy that the spinal artery arises from the vertebral arteries, and is reinforced by segmental spinal arteries. It is rather to be considered that this artery arises from the segmental spinal arteries, and anastomoses with, or is reinforced by, the vertebrals.

The term median dorsal is suggested for the artery present in places in the median dorsal line of the spinal cord.

My thanks are due to Dr. Richard E. Scammon for his constant interest in the progress of this work, and for his many helpful criticisms.

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August, 1914

PERSISTENT ARTERIAE BRACHII SUPERFICIALIS, ANTIBRACHII SUPERFICIALIS ET MEDIANA

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From the Institute of Anatomy, University of Minnesota

ONE FIGURE

An unusual artery found in the left arm of a man of thirty-seven years seems worthy of record.

The vessel emerges from the axillaris midway between the aa. subscapularis and thoracalis lateralis, on the median side. It runs in the deep fascia anterior and medial to the a. axillaris, the a. brachialis and the n. medianus, almost to the middle of the humerus, where it crosses the a. brachialis and the n. medianus, to enter the m. biceps brachii from beneath, through two large divisions.

It gives off two small cutaneous rami in the lower axillary and upper brachial regions. In size the artery is about two-thirds that of the normal subscapularis until it reaches the biceps muscle. At this point it gives rise to a small ramus almost at right angles to it. This courses lateral and anterior to the brachial artery in the deep fascia, becomes superficial at the elbow, and continues anterior to the ulna, to the palm. Here it enters into formation of the arcus volaris superficialis, together with the a. ulnaris. The arch has no connection with the a. radialis.

There is no ramus of the a. ulnaris or a. interossea which may be called an a. mediana. The a. mediana described in this paper has no relation to the n. medianus, which is placed deep in the forearm.

The embryological significance of the artery in question may be derived from Müller's[1] figure of the arteries in the arm of an

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[1] Müller '03, Anat. Hefte, Bd. 22, Taf. 25-26, fig. 9.

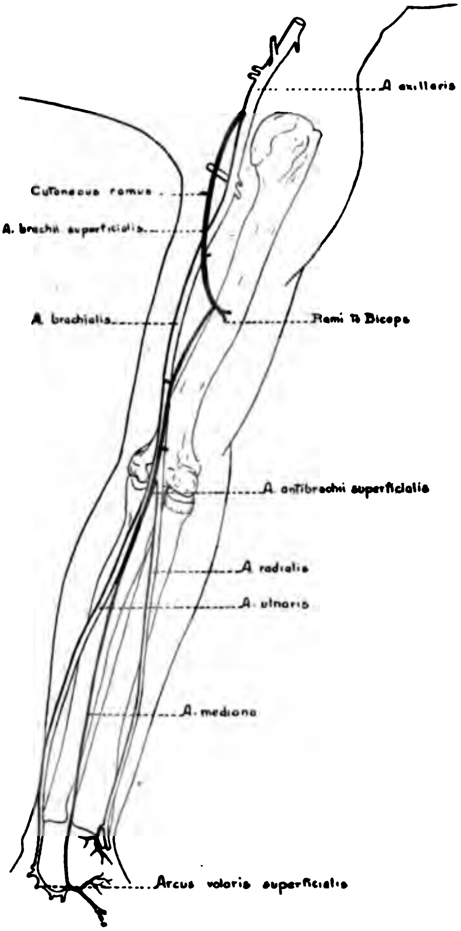

Fig. 1 Persistent arteriæ brachii superficialis, antibrachii superficialis et mediana. The rami of the aa. brachialis, radialis and ulnaris are not shown in the figure, as they are normal except for the two discrepancies noted.

11.7 mm. human embryo ('03). From this figure it would seem that we have a persistent a. brachialis superficialis, giving rise to an a. antibrachii superficialis, which becomes an a. mediana, but all anastomoses with the a. brachialis have been lost. As stated by some texts of anatomy, this condition is one that is quite rarely found.

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24. *On the development of the digitiform gland in Squalus acanthias.*

E. R. Hoskins, Institute of Anatomy, University of Minnesota.

The digitiform gland in Aqualus is evidenced first by a slight thickening of the entoderm of the dorso-lateral border of the gut just posterior to the spiral valve. This may be seen in embryos 15 mm. in length, especially in those sectioned longitudinally. The thickening soon pushes laterally to form a hollow bud which turns and grows anteriorly along the gut. The form of the curved portion at the point of emergence from the gut always persists so that in older stages and in the adult this portion which becomes the duct of the gland enters both the intestine and the digitiform gland anteriorly.

In the stage of 28 mm. it may be seen that from the main part of the gland small buds resembling the original form of the gland grow laterally on all sides. These buds become tubules extending laterally and slightly posteriorly. They in turn give rise to secondary tubules which in time form irregular groups opening into the primary tubules. This condition is to be found throughout development, the gland becoming a compound tubular structure, the secondary tubules arising from the primary, close to the main lumen of the gland.

As the gland develops, it carries the mesentery of the intestine with it and is thus supported from the dorsal wall of the body cavity.

The entoderm of the digitiform gland is composed at first of four layers of low columnar or cuboidal cells with elongated nuclei, being similar to the entoderm of the gut from which it develops. As the gland increases in length, the epithelium is gradually reduced to one layer in thickness. Its primary and secondary tubules both arise as structures of an epithelium of one layer of cells. At the points of greatest growth, namely, at the distal ends of the tubules the nuclei are wider and shorter than along the main lumen, often being spherical.

The epithelium lining the main or central lumen later thickens giving us a structure of two layers of columnar cells with rounded nuclei in the full-term fetus and of four layers in the adult.

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INTRODUCTION

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ANATOMY, as the term is usually employed, denotes the study of the structure of the human body. Properly, however, it has a much wider significance, including within its scope not man alone, but all animal forms, and, indeed, plant forms as well; so that, when its application is limited to man, it should be qualified by the adjective human. *Human Anatomy*, then, is the study of the structure of the human body, and stands in contrast to, or rather in correlation with, *Human Physiology*, which treats of the functions of the human body, the two sciences, Anatomy and Physiology, including the complete study of man's organization and functional activities.

In the early history of the sciences these terms sufficed for all practical needs, but as knowledge grew, specialization of necessity resulted and new terms were from time to time introduced to designate special lines of anatomical inquiry. With the improvement of the microscope a new field of anatomy was opened up and the science of *Histology* came into existence, assuming control over that portion of Anatomy which dealt with the minuter details of structure. So, too, the study of the development of the various organs gradually assumed the dignity of a more or less independent study known as *Embryology*, and the study of the structural changes due to disease was included in the science of *Pathology;* so that the term Anatomy is sometimes limited to the study of the macroscopic structure of normal adult organisms.

It is clear, however, that the lines of separation between Anatomy, Histology, Embryology, and Pathology are entirely arbitrary. Microscopic anatomy necessarily grades off into macroscopic anatomy; the development of an organism is a progressive process and the later embryonic or fœtal stages shade gradually into the adult; and structural anomalies lead insensibly from the normal to the pathological domains. Furthermore it is found that in its individual development the organism passes through stages corresponding to those of its ancestry in evolution; in other words, Ontogeny repeats Phylogeny. A comprehensive study of Anatomy must therefore include more or less of the other sciences, and since an appreciation of the significance of structural details can only be obtained by combining the studies of Anatomy, including Histology and Embryology, and since, further, much light may be thrown on the significance of embryological stages by comparative studies, Anatomy, Embryology, and Comparative Anatomy form a triumvirate of sciences by which the structure of an organism, the significance of that structure, and the laws which determine it are elucidated. For this combination it is convenient to have a single term, and that which is used is *Morphology*, a word meaning literally the science of form.

In morphological comparisons, the term *homology* denotes similarity of structure, due to a common origin in the evolution of organs or parts; while *analogy* denotes merely physiological correspondence in function. Thus the arm of man and the wing of a bird are homologous, but not analogous, structures; on the other hand, the wing of a bird and the wing of an insect are analgous, but not homologous. *Serial homology* refers to corresponding parts in successive segments of the body.

Nomenclature.—Formerly there was much confusion in the anatomical nomenclature, due to the multiplicity of names and the lack of uniformity in using them. Various names were applied to the same organs and great diversity of usage prevailed, not only between various countries, but also even among authors of the same country. Recently, however, a great improvement has been made by the general adoption of an international system of anatomical nomenclature. This system was first adopted by the German Anatomical Society at a meeting in Basel, in 1895, and is hence called the Basel Nomina Anatomica, or briefly, the BNA. The BNA provides each term in Latin form, which is especially desirable for international usage. Each nation, however, is expected to translate the terms into its own language, wherever it is deemed preferable for everyday usage. Thus in the present work the Anglicised form of the BNA is generally used. Where not identical, however, the Latin form is added once for each term in a place convenient for reference, and is designated by enclosure in brackets [ ]. Where necessary the older terms have also been added as synonyms.

The Commission by whom the BNA was prepared included eminent anatomists representing various European nations. The work of the Commission was very thorough and careful, and extended through a period of six years. Among the guiding principles in the difficult task of selecting the most suitable terms were the following: (1) Each part should have one name only. (2) The names should be as short and simple as possible. (3) Related structures should have similar names. (4) Adjectives should be in opposing pairs. A few exceptions were found necessary, however.

On account of its obvious merits, the BNA system has been generally adopted throughout the civilised world, and the results are very satisfactory. Comparatively few new terms have been thereby introduced, over 4000 of the 4500 names in the BNA corresponding almost exactly to older terms already in use by the English-speaking nations. Certain minor defects in the system have been criticised; but these are outweighed by the advantages of this uniform system.

Abbreviations.—Certain frequently used words in the BNA are abbreviated as follows: a., arteria (plural, aa., arteriæ); b., bursa; g., ganglion; gl., glandula; lig., ligamentum (plural, ligg., ligamenta); m., musculus (plural, mm., musculi); n., nervus (plural, nn., nervi); oss., ossis (or ossium); proc., processus; r., ramus (plural, rr., rami); v., vena (plural, vv., venæ).

Terms of position and direction.—The exact meaning of certain fundamental terms used in anatomical description must be clearly understood and kept in mind. In defining these terms, it is supposed that the human body is in an upright position, with arms at the sides and palms to the front.

The three **fundamental planes** of the body are the sagittal, the transverse and the frontal. The vertical plane through the longitudinal axis of the trunk, dividing the body into right and left halves, is the *median* or *mid-sagittal* plane; and any plane parallel to this is a *sagittal* plane. Any vertical plane at right angles to a sagittal plane, and dividing the body into front and rear portions is a *frontal* (or coronal) plane. A plane across the body at right angles to sagittal and coronal planes is a *transverse* or *horizontal* plane.

Terms pertaining to the front of the body are *anterior* or *ventral*; to the rear, *posterior* or *dorsal*; upper is designated as *superior* or *cranial*; and lower as *inferior* or *caudal*.

The term *medial* means nearer the mid-sagittal plane, and *lateral*, further from that plane. These terms should be carefully distinguished from internal (inner) and external (outer), which were formerly synonymous with them. *Internal*, as now used (BNA), means deeper, i. e., nearer the central axis of the body or part; while *external* refers to structures more superficial in position. *Proximal*, in describing a limb, refers to position nearer the trunk; while *distal* refers to a more peripheral position.

Adverbial forms are also employed, e. g., anteriorly or ventrally (forward, before); posteriorly or dorsally (backward, behind); superiorly or cranially (upward, above); and inferiorly or caudally (downward, below).

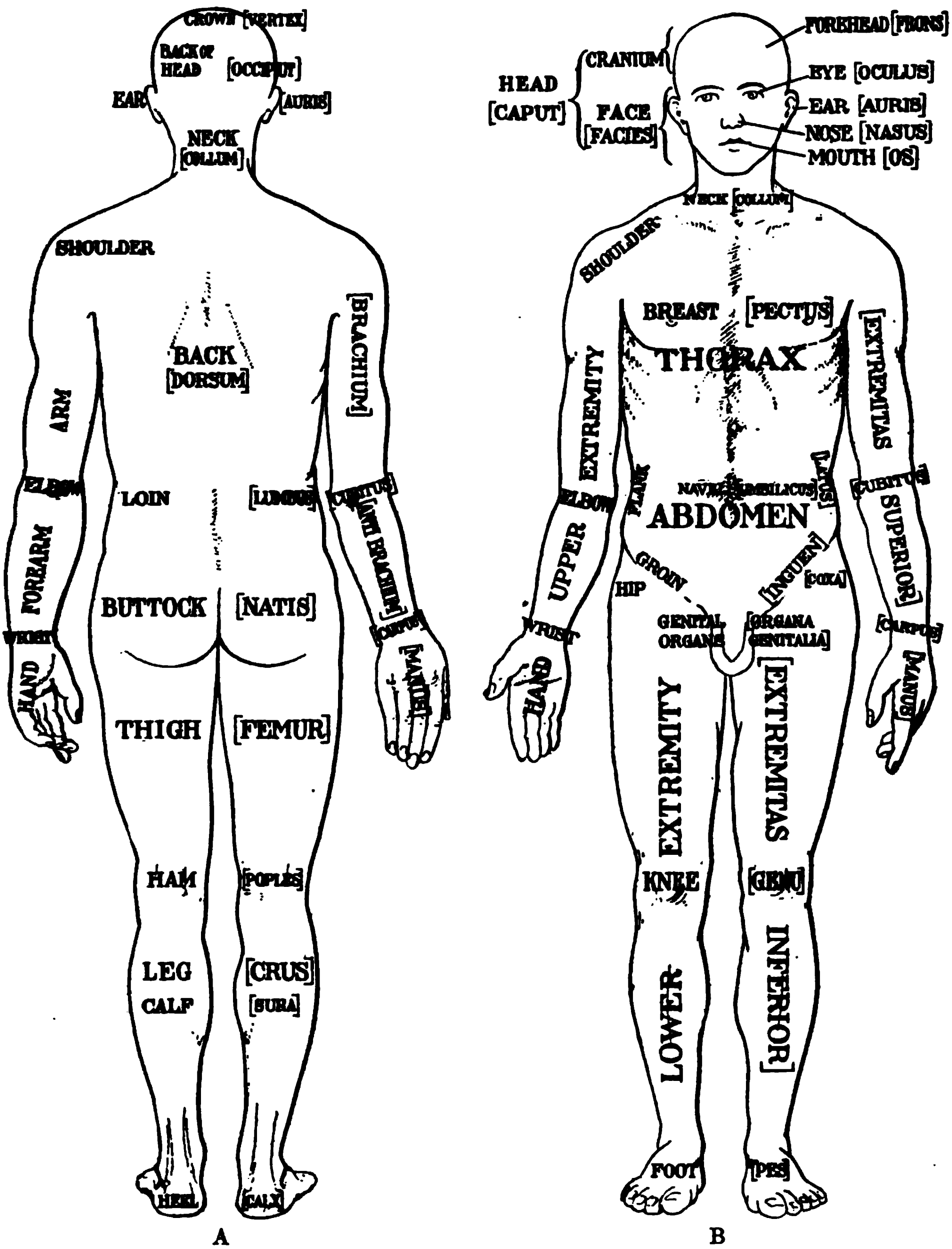
It should also be noted that the terms ventral, dorsal, cranial and caudal are independent of the body posture, and therefore apply equally well to corresponding surfaces of vertebrates in general with horizontal body axis. On this account these terms are preferable, and will doubtless ultimately supplant the terms anterior, posterior, superior and inferior.

The discrimination in the use of several similar terms of the BNA should also receive attention. Thus *medianus* (median) refers to the median plane. *Medialis* (medial) means nearer the median plane and is opposed to lateral, as above stated. *Medius* (middle) is used to designate a position between anterior and posterior, or between internal and external. Between medialis and lateralis, however, the term *intermedius* is used. Finally, *transversalis* means transverse to the body axis; *transversus*, transverse to an organ or part; and *transversarius*, pertaining to some other structure which is transverse.

Parts of the body.—The primary divisions of the human body (fig. 1) are the head, neck, trunk and extremities. The *head* [caput] includes *cranium* and *face* [facies]. The *neck* [collum] connects head and trunk. The *trunk* [truncus] includes *thorax*, *abdomen*, and *pelvis*. The *upper extremity* [extremitas superior] includes *arm* [brachium], *forearm* [antibrachium], and *hand* [manus]. The *lower extremity* [extremitas inferior] includes *thigh* [femur], *leg* [crus], and *foot* [pes].

Each of the parts mentioned has further subdivisions, as indicated in fig. 1. The cranium includes: *crown* [vertex]; *back* of the head [occiput]; *frontal* region [sinciput], including *forehead* [frons]; *temples* [tempora]; *ears* [aures], including *auricles* [auriculæ].

Fig. 1.—Parts of the Human Body. A, Posterior view. B, Anterior view.



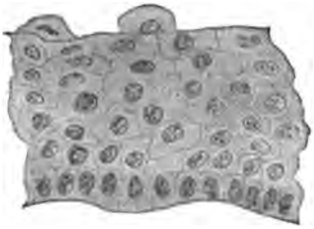
The face includes the regions of the *eye* [oculus], *nose* [nasus], and *mouth* [os], the subdivisions of which will be given later under the appropriate sections.

The thorax includes: *breast* [pectus]; *mammary gland* [mamma]; and *thoracic cavity* [cavum thoracis]. The *back* [dorsum] includes the vertebral column [columna vertebralis]. The abdomen includes: *navel* [umbilicus]; *flank* [latus]; *groin* [inguen]; *loin* [lumbus]; and the *abdominal cavity* [cavum abdominis]. The pelvis includes: *pelvic cavity* [cavum pelvis]; *genital organs* [organa genitalia], *buttocks* [nates], separated by a cleft [crena ani] at the *anus*. The *hip* [coxa] connects the pelvis with the lower extremity.

In the lower extremity, the thigh is joined to the leg by the *knee* [genu]. The foot includes: *heel* [calx]; *sole* [planta]; *instep* [tarsus]; *metatarsus;* and five *toes* [digiti I–V], including the *great toe* [hallux] and *little toe* [digitus minimus].

The upper extremity is joined to the thorax by the *shoulder*. The arm is joined to the forearm at the *elbow* [cubitus]. The hand includes: *wrist* [carpus]; *metacarpus*, with *palm* [vola or palma] and *back* [dorsum manus]. The five *fingers* [digiti I–V] include: *thumb* [pollex], *index* finger [index]; *middle* finger [digitus medius]; *ring* finger [digitus annularis] and *little* finger [digitus minimus].

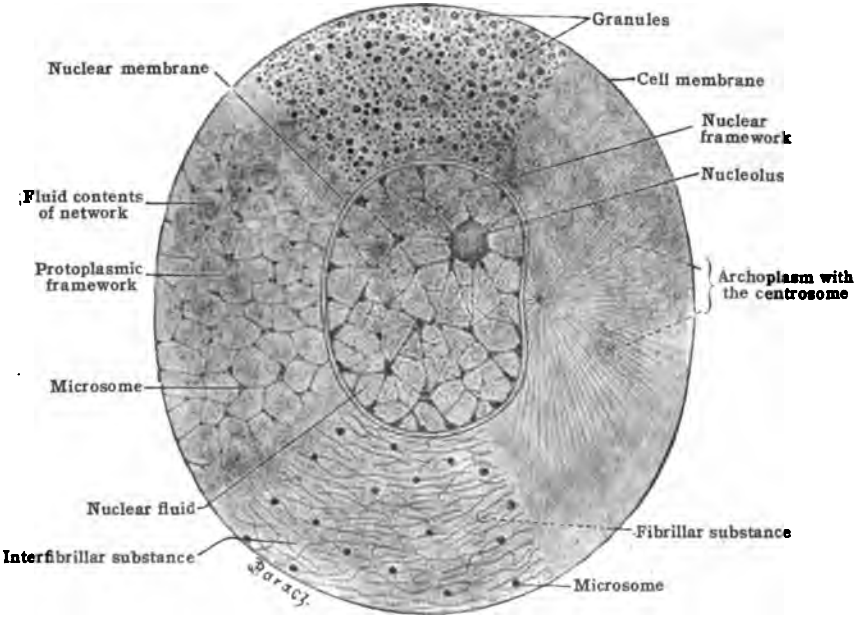
FIG. 2.—SECTION OF THE EPIDERMIS OF A FINGER, FROM A HUMAN EMBRYO OF 10.2 CM.



Organ-systems.—Each of the various parts of the body above outlined is composed of various *organs*, and the groups of related organs make up *organ-systems*.

The various organ-systems are treated as special branches of descriptive anatomy. The study of the *bones* is called *osteology;* of the *ligaments* and *joints*, *syndesmology* (or arthrology); of the *vessels*, *angiology*; of the *muscles*, *myology;* of the *nervous system*, *neurology*; and of the *viscera*, *splanchnology*. Further subdivisions are also made. The viscera, for example, include the *digestive* tract, *respiratory* tract, *urogenital* tract, etc.

FIG. 3.—DIAGRAM OF A TYPICAL CELL. (Szymonowicz.)



Tissues and cells.—The body, as above stated, has various parts, each of which may be subdivided into its component systems and organs. A further analysis reveals a continued series of structural units of gradually decreasing complexity. Thus each organ is found to consist of a number of *tissues* (epithelial, connective, muscular or nervous). Finally, each tissue is composed of a group of similar units called *cells* (figs. 2, 3) which are the ultimate structural units of the body. The body may therefore be regarded as composed of myriads of cell units, organized into units of gradually increasing complexity, very much as a social community is composed of individuals organized into trades, municipalities, etc.

Most of the individual tissues can be recognized by their gross appearance. In fact, the principal tissues were first demonstrated by Bichat through skilful dissection, maceration, etc., and without the aid of the microscope. The cellular structure of the tissues was later discovered by Schwann in 1839.

Each cell (fig. 3) is composed of a material called *protoplasm*, a viscid substance variable in appearance and exceedingly complex in chemical composition. It readily breaks down into simpler chemical compounds, whereby energy (chiefly in the form of heat and mechanical energy) is liberated. It has also the power of absorbing nutritive material to build up and replace what was lost. Its decomposition results from stimuli of various kinds, and hence it is said to be irritable. The mechanical energy which it liberates is manifested by its contractility, especially in the muscle cells. It excretes the waste products produced by its decomposition. Each cell has the power, under favourable conditions, of reproducing itself by division. Protoplasm presents, in short, all the forms of activity manifested by the body as a whole; and, indeed, the activities of the body are the sum of the activities of its constituent cells.

In the protoplasm of each cell is a specially differentiated portion, the *nucleus* (fig. 3). The nucleus plays an important part in regulating the activities of the *cytoplasm*, the general protoplasm of the cell body. The nucleus differs from the cytoplasm both structurally and chemically, and contains a very important substance, *chromatin*, which during cell division is aggregated into a definite number of masses called *chromosomes*. The cytoplasm of actively growing cells also contains the *archoplasm* and *centrosome*, structures of importance in the process of cell division. Further details concerning the cells and tissues may be found in the text-books of cytology and histology.

In earlier days Human Anatomy was almost entirely a descriptive science, but little attention being paid to the significance of structure, except in so far as it could be correlated with physiological phenomena as they were at the time understood. In recent years attention has been largely paid to the morphology of the human body and much valuable information as to the meaning of the structure and relations of the various organs has resulted. Since the form and structure of the body are the final result of a series of complicated developmental changes, the science of Embryology has greatly contributed to our present knowledge of human Morphology; and, accordingly, a brief sketch of some of the more important phases of morphogenesis will form a fitting introduction to the study of the adult.

References.—*General:* For looking up the literature upon any anatomical topic, the best guide is the "Jahresbericht ueber die Fortschritte der Anatomie und Entwicklungsgeschichte," which contains classified titles and brief abstracts of the more important papers in gross anatomy, histology and embryology. Other useful aids are the "Zentralblatt fuer normale Anatomie," the "Index Medicus" and the catalogue of the Surgeon Genera 's Library of the War Dep't. (Washington, D. C.). The latter two contain titles only, but cover the whole field of medicine. The "Concilium Bibliographicum" also provides a convenient card-index system of references for the biological sciences, including Anatomy.

For *nomenclature:* His, Archiv f. Anat., 1895 (BNA system); Barker, Anatomical Nomenclature. *Cells and tissues:* Wilson, The Cell; Hertwig, Zelle und Gewebe (also English transl.); Schaefer, Microscopic Anatomy (in Quain's Anatomy, 11th ed.); Heidenhain, Plasma und Zelle.

SECTION I

MORPHOGENESIS

REVISED FOR THE FIFTH EDITION

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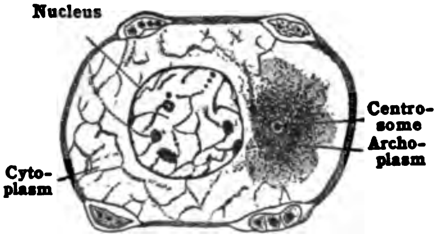
CHANGE is a fundamental characteristic of all living things. The human body during its life cycle accordingly passes through various phases of form and structure. In the earliest embryonic phases of development the changes are very rapid, decreasing in rapidity during the later fœtal stages, but continuing at a diminishing rate throughout infancy, childhood and youth up to the adult. Following the acme of maturity, changes continue which lead gradually to senescence and final death of the body.

This cycle of change in the body depends upon similar changes in its various component organs, each having its own characteristic life cycle. In a few of the organs this cycle is very short, as in some of the organs of the embryo (e. g., mesonephros). Other organs persist only during childhood (e. g., thymus); while the majority continue, with varying degrees of change, throughout postnatal life. The final death of the body is due to the breakdown of some of the essential organs.

A further analysis reveals the fact that the characteristic life cycles of the organs depend ultimately upon similar changes in their constituent tissues and cells. Every cell has a definite life cycle, an early period characterised by rapid and vigourous changes, later periods of differentiation and maturity, followed by stages of degeneration and death. This cycle of cell changes has been designated by Minot as *cytomorphosis*.

Growth.—Associated with the process of cell differentiation (cytomorphosis), and even more important as a factor in the morphogenesis of the body, is the process of *growth*. The developmental changes in form and structure of the body are due largely to the unequal growth of its various parts. Growth, like other changes in the body and its parts, depends ultimately upon the characteristics of the constituent cells.

FIG. 4.—THE OVUM OF A NEW-BORN CHILD, WITH FOLLICLE CELLS. (After Mertens.)



The cell changes during growth may be grouped under two heads. The first, or growth proper, involves merely the *enlargement* (hypertrophy) of the individual cells and intercellular products. The second includes the *multiplication* (hyperplasia) of the cells, which is accomplished by mitotic division. Cell division is necessary in cell growth, for otherwise the cell would soon reach a size where its surface (for nutritive, respiratory and excretory purposes) would be inadequate for its mass. In general, however, cell division is most active in the earlier embryonic periods, during which the cells remain small. Later, cell division diminishes or ceases, and growth is due chiefly to enlargement of the cells already present. It is also during the later period, when the cells have ceased rapid division, that the process of cell differentiation and tissue formation is most marked.

The principle of the *ratio of surface to mass* often applies to the growing organs as well as to the individual cells. To maintain the necessary ratio, the surface area is increased by the formation, through localised unequal growth, of *projections* (e. g., villi or folds) or *invaginations* (e. g., glands) from surfaces. Innumerable modifications of this principle occur throughout the process of morphogenesis.

FIG. 5.—OVUM FROM OVARY OF A WOMAN THIRTY YEARS OF AGE. *cr*, corona radiata. *n*, nucleus. *y*, yolk. *p*, clear protoplasmic zone, *ps*, perivitelline space. *zp*, zona pellucida. (McMurrich's Embryology, from Nagel.)

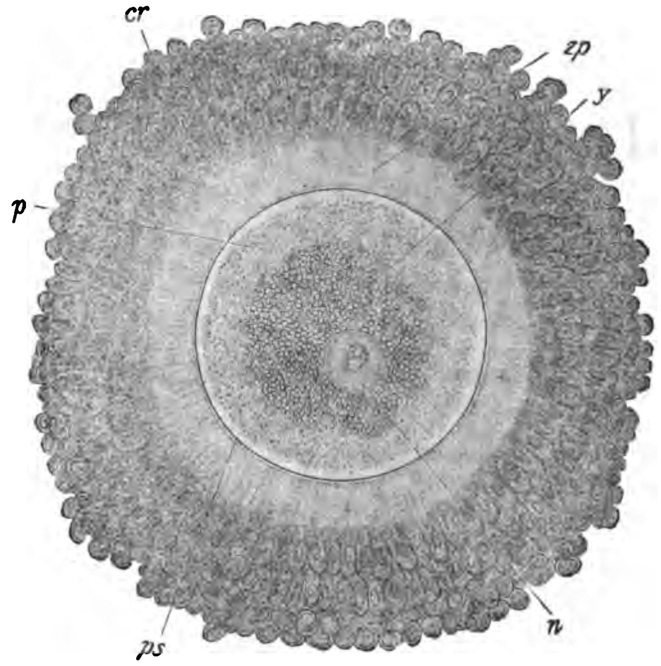
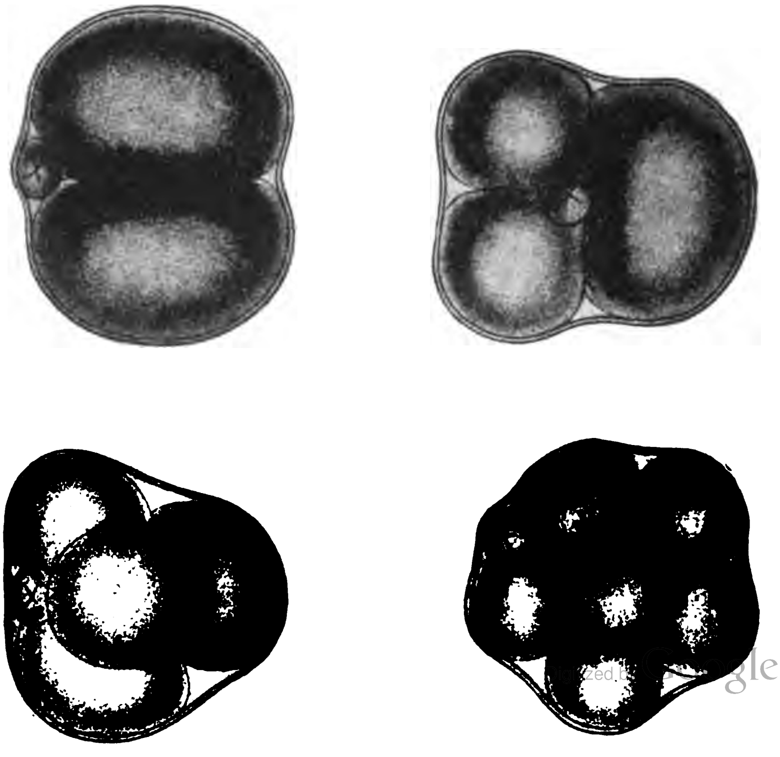


FIG. 6.—STAGES OF SEGMENTATION IN THE OVUM OF THE MOUSE. *x*, polar body. (McMurrich's Embryology, from Sobotta.)



While the present work deals primarily with the adult human organism in the stage of maturity, reference is made also to its changes according to age. Although these changes for the various systems of organs are described under the appropriate sections, it is desirable to consider first some of the more fundamental features pertaining to the body as a whole. This applies particularly to the earlier embryonic period, which includes the more general phases of morphogenesis. No attempt will be made to describe fully the process of development, the details of which are to be found in text-books of embryology.

Segmentation of the ovum.—The human body, like all living organisms, arises from a single cell, the egg-cell or *ovum.* An early stage in the development of the ovum is shown in fig. 4, and a later stage, approaching maturity, in fig. 5. The mature human ovum is about 0.2 mm. in diameter. In the uterine (Fallopian) tube, the fertilised ovum undergoes segmentation, the various stages of which are represented in figs. 6 and 7.

FIG. 7.—DIAGRAM OF SECTION THROUGH A MAMMALIAN OVUM AT THE MORULA STAGE.

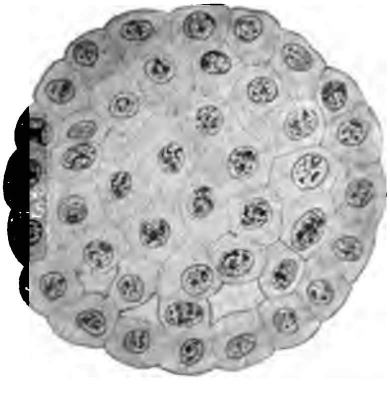
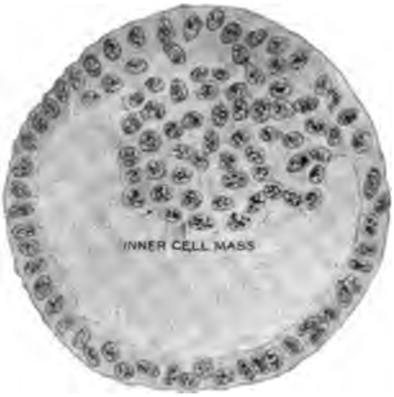


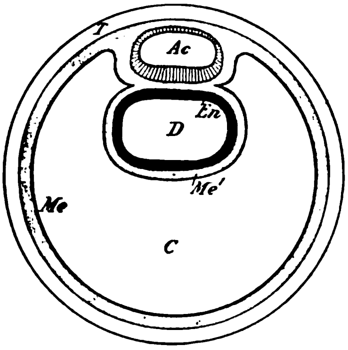
FIG. 8.—DIAGRAM OF SECTION OF A MAMMALIAN OVUM SHOWING THE INNER CELL MASS.



While the processes of maturation, fertilisation and segmentation have not as yet been observed in the human ovum, the evidence of comparative anatomy makes it very probable that in all essential respects these processes are like those found in other mammals. As a result of the successive divisions of the ovum in segmentation, a spherical mass of cells, the *morula* (fig. 7) is formed. In this mass, an excentric cavity forms (fig. 8) whereby the mass is transformed into a hollow vesicle. The wall of this vesicle is probably formed throughout the greater part of its extent by a single layer of cells; but at one point of the circumference there is a group of cells termed the *inner cell mass* (fig. 8). Probably about this time the ovum enters the uterine cavity, and through the activity of the outer layer of cells (*trophoblast*) becomes embedded in the uterine mucosa.

Formation of the embryonic disc and germ layers.—In the earliest human embryos which have been described, development has already proceeded beyond the stage represented by fig. 8, and has reached that of fig. 9. Within the inner cell mass, two cavities have appeared. The more superficial (ac) is the *amniotic*

FIG. 9.—DIAGRAM SHOWING THE RELATIONS OF THE GERM LAYERS IN AN EARLY EMBRYO. *Ac*, amniotic cavity, lined by ectoderm. *D*, yolk-sac, lined by endoderm (*En*). *Me,Me'*, mesoderm, *C*, extra-embryonic cœlom. *B*, chorion. *T*, trophoblast. (McMurrich.)

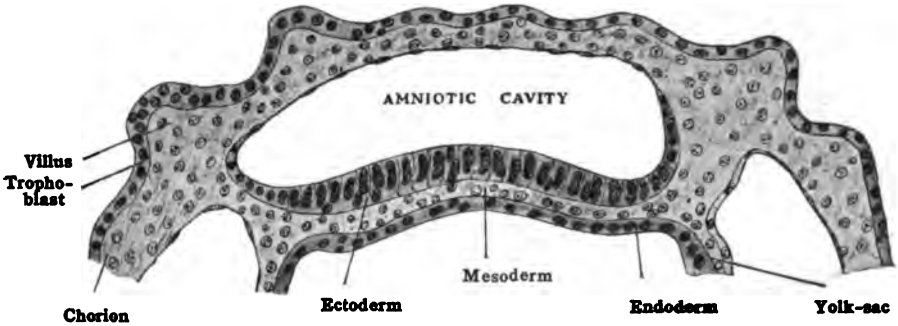


cavity; the deeper (D) is the cavity of the *yolk-sac;* while between them is a plate of cells forming the *embryonic disc.* The embryonic disc (figs. 9 and 10) contains three layers of cells,—the fundamental *germ layers,*—*ectoderm* (Ec), *endoderm* (En), and *mesoderm.*

The germ layers of the embryonic disc are of prime importance in the development of the body. From the *ectoderm,* which lies next to the amniotic cavity and represents the upper (later outer) germ layer, are derived the epidermis and the entire nervous system. From the *endoderm,* which lies next to the yolk-sac, and represents the lower (later inner) germ layer, is derived the epithelial lining of the digestive mucosa and its derivatives. From the *mesoderm,* or middle germ layer, is differentiated the remainder of the body, including the skeletal and supporting tissues, vascular system, muscle and most of the urogenital organs.

The germ layers also extend beyond the embryonic disc, as shown in figs. 9 and 10. The *yolk-sac* is made up of a lining of endoderm and an outer layer of mesoderm. The *amnion,* which later becomes separated from the chorion, is composed of mesoderm lined by endoderm. The outer cell layers form the *chorion,* which likewise shows two layers, the outermost of which (trophoblast) is ectoderm, the inner, mesoderm. In fig. 10 the chorion is beginning to send out root-like projections (villi) which invade the uterine mucosa.

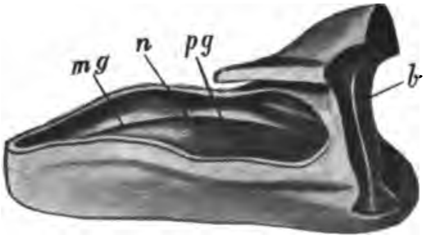
Fig. 10.—Diagram of Section of a Mammalian Ovum showing the Embryonic Disc, Amniotic Cavity and the Germ Layers.



It is thus noteworthy that of the cells derived from the ovum relatively only a few—those of the embryonic disc—enter directly into the formation of the body. The yolk-sac, a rudimentary organ of phylogenetic significance, is later chiefly absorbed, although the proximal portion may enter slightly into the formation of the intestinal wall. The amnion is a protective membrane, while the chorion forms the fœtal part of the placenta.

Development of the embryonic disc.—When first formed, the surface of the embryonic disc shows no trace of differentiation. A slightly later but still comparatively early stage in its development is shown in fig. 11. It is here viewed from above, the amnion having been removed. The disc is an elliptical plate, whose long axis represents the mid-line of the embryo. Near the center is a small rounded depression, the *primitive pit.* Extending backward (toward the tail end of the embryo) from this is a dark line, the *primitive streak,* corresponding to a groove, the *primitive groove.* Extending forward from the primitive pit is an indistinct wide shallow groove, the *neural groove.*

Fig. 11.—Model Showing the Embryonic Disc from an Embryo 1.17 mm. In Length. Viewed from above and laterally, the roof of the amniotic cavity having been removed. *n,* primitive pit (neurenteric canal). *pg,* primitive groove. *mg,* neural groove. *b,* body-stalk. (McMurrich, from Frassi.)

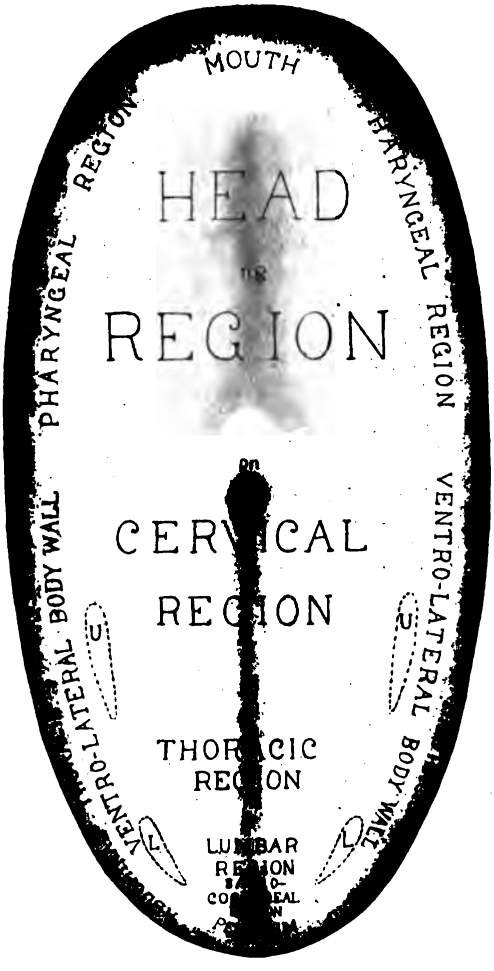


At an earlier stage, the primitive streak extends further forward, possibly to the anterior end of the embryonic disc (Spee). The primitive streak and groove probably correspond to the fused lips of a primitive blastopore. They represent a centre of proliferation from which the mesoderm is budded off from the ectoderm and spreads out to form the middle germ layer of the embryonic disc.

At the anterior end of the primitive streak this proliferation extends forward as a plate of cells, the so-called *'head process.'* The axial portion of this process is the anlage of the *notochord*, the embryonic skeletal axis. It contains a canal, which opens into the primitive pit. The notochordal anlage soon fuses with the underlying endoderm, and its canal forms the transient *neurenteric canal*.

In the mid-line anterior to the primitive streak there appears the shallow *neural groove* (fig. 11), corresponding to a thickened plate of ectodermic cells, the *neural plate*. The neural groove is slightly forked at its posterior extremity, in the region of the *primitive node* (Hensen's node), which forms the dorsal lip of the primitive pit. As development proceeds, the neural plate extends posteriorly, and the primitive pit is accordingly shifted backward, the corresponding part of the primitive groove being converted into 'head process.' The primitive streak thus becomes progressively shortened (cf. figs. 11 and 13).

FIG. 12.—TOPOGRAPHY OF THE EMBRYONIC DISC. DIAGRAM OF RELATIONS AT THE LENGTH OF ABOUT 1 MM. *ng*, neural groove. *pn*, primitive node. *pp*, primitive pit. *U*, upper limb. *L*, lower limb.



Topography of the embryonic disc.—Although only slight signs of differentiation are visible in the embryonic disc at the stage shown in fig. 11, it is already possible to map out more or less definite areas corresponding to all the various regions of the future body, as shown in fig. 12.

Beginning anteriorly, the head region is relatively enormous in size, occupying at this time the entire portion in front of the primitive pit and forming about half of the entire disc. The cervical, thoracic, lumbar and sacro-coccygeal regions appear successively smaller, approaching the posterior end ('tail bud') of the primitive streak. It is also a striking fact that the future dorsal region of the body wall, corresponding to the central portion of the disc, along each side of the mid-line, is now larger than the ventro-lateral regions, which occupy a relatively narrow area around the periphery of the disc.

The topography of the germinal areas in the embryonic disc shown in fig. 12 is based partly upon a study of the succeeding stages of development, and partly upon the results of experiments upon the germinal disc in lower forms, especially in the chick (Assheton, Peebles, Kopsch).

Law of developmental direction.—In the relative size of the various embryonic areas is foreshadowed what may be termed the law of direction in development. In general it is found that development (including growth and differentiation) in the long axis of the body appears first in the head region and progresses toward the tail region. Similarly in the transverse plane development begins in the mid-dorsal region and progresses latero-ventrally (in the limbs, proximo-distally). These principles are of great importance in morphogenesis.

Fig. 13.—Human Embryo 1.54 mm. long. Viewed from above, the roof of the amniotic cavity having been removed. (Minot, after Graf Spee.)

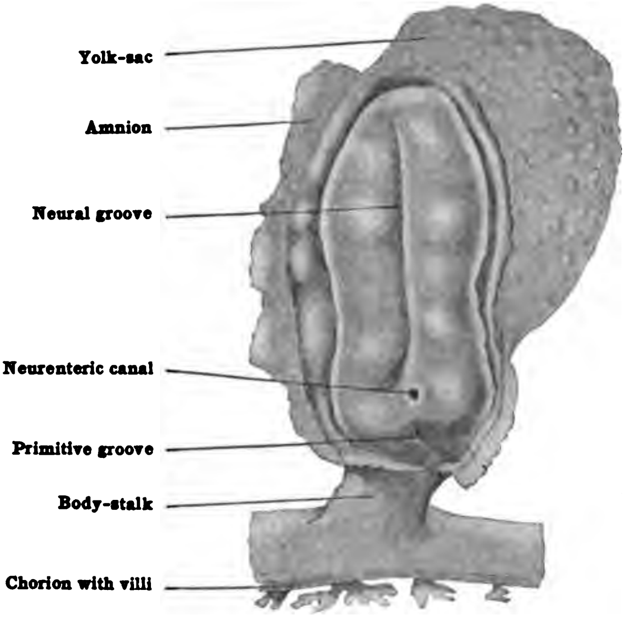
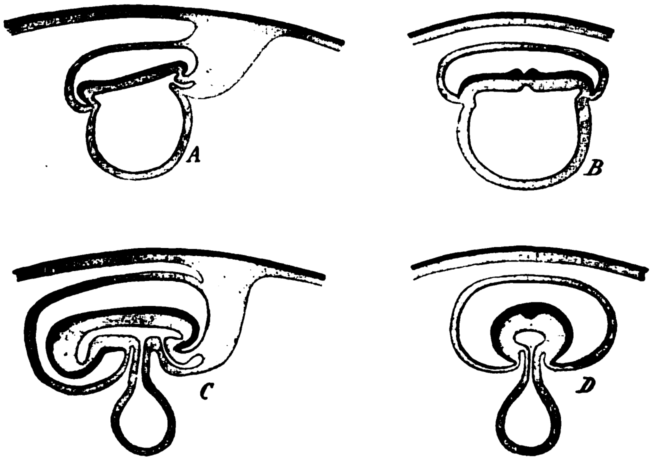


Fig. 14.—Diagrams Showing the Constriction of the Embryo from the Yolk-sac. *A* and *C*, longitudinal sections; *B* and *D*, corresponding cross-sections. (McMurrich.)



The law of developmental direction is also probably of phylogenetic significance. The *cranio-caudal* direction of development is in accordance with the theory that the head is the most primitive portion of the body, and hence precocious in development. The trunk is perhaps a secondary acquisition, hence arising as an extension of the primitive head region.

The *dorso-ventral* direction of development, together with the plate-like form of the embryonic disc, has a different phylogenetic significance. Both are probably inherited from an ancestral type with a yolk-laden ovum. In such an ovum, with the meroblastic type of segmentation, the flattened embryonic disc gradually spreads from the dorsal surface in a ventral direction around the underlying yolk-mass.

Derivation of body tube from embryonic disc.—The primary result of the precocious growth in the dorsal region of the embryonic disc is the conversion of the disc into the body tube, curved ventrally in its long axis (fig. 14).

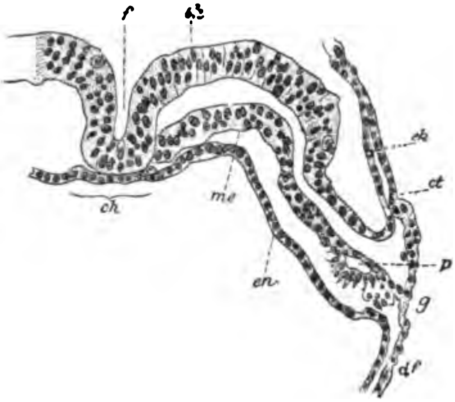
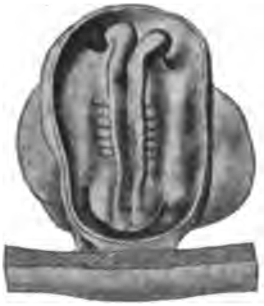


Fig. 15.—Portion of Cross Section of the Embryo shown in Fig. 13. *ch*, notochord. *ct*, somatic mesoderm. *df*, splanchnic mesoderm. *g*, junction of extra-embryonic somatic and splanchnic mesoderm. *ek*, ectoderm. *en*, endoderm. *me*, embryonic mesoderm. *f*, neural groove, *p*, beginning of embryonic cœlom (pericardial cavity). (Minot, after Graf Spee.)

As a result of the more rapid expansion of the germ layers (especially the ectoderm) near the mid-line, the dorsal surface of the embryonic disc in general becomes convex, with a depression laterally (where growth is less rapid) forming a groove at the line of attachment of the amnion (figs. 11, 12, 13, 14 B). The unequal growth in the germ layers is clearly evident in the cross section shown in fig. 15. By a continuation of this process, the margins of the embryonic disc become still further depressed and finally folded in ventrally so as to transform the disc into a tube (fig. 14 D). Similarly, by a more rapid expansion of the dorsal layer of the disc in the longitudinal axis, the head and tail ends of the disc are folded and tucked in ventrally, and the primitive body tube is thus correspondingly curved in its long axis (figs. 14 A, 14 C).

Fig. 16.—Model of Human Embryo 1.8 mm. Long. Viewed from above, the roof of the amniotic cavity having veen removed. Near the caudal end of the neural groove, the primitive pit (opening of neurenteric canal) is visible. The primitive somites are appearing in the occipital region, the fourth corresponding to the boundary between head and neck. (McMurrich, from Keibel and Elze.)



The embryonic disc is thus converted into a tube composed of an outer layer of ectoderm, a middle layer of mesoderm and an inner layer of endoderm. The yolk-sac now presents an expanded *yolk-vesicle* lined by endoderm which is still continuous through the constricted *yolk-stalk* with the endoderm lining the primitive *enteric cavity* (fig. 14 C). The enteric cavity (or archenteron) has a blind tubular prolongation (fore gut) into the head region, and another (hind gut) into the tail region. From the latter a slender diverticulum, the *allantois*, extends into the body stalk (later the umbilical cord). The allantois is an organ of phylogenetic importance, with which the urinary bladder is later connected.

Formation of the neural tube.—The principle of unequal growth applies to the formation not only of the body as a whole, but also of its constituent parts. Thus the anlage of the nervous system arises from the ectoderm as a wide groove whose edges (neural ridges) by local growth are folded upward so as to meet in the mid-line where they fuse, thus transforming the groove into the *neural tube* (figs. 11, 12, 13, 15, 16, 17, 18).

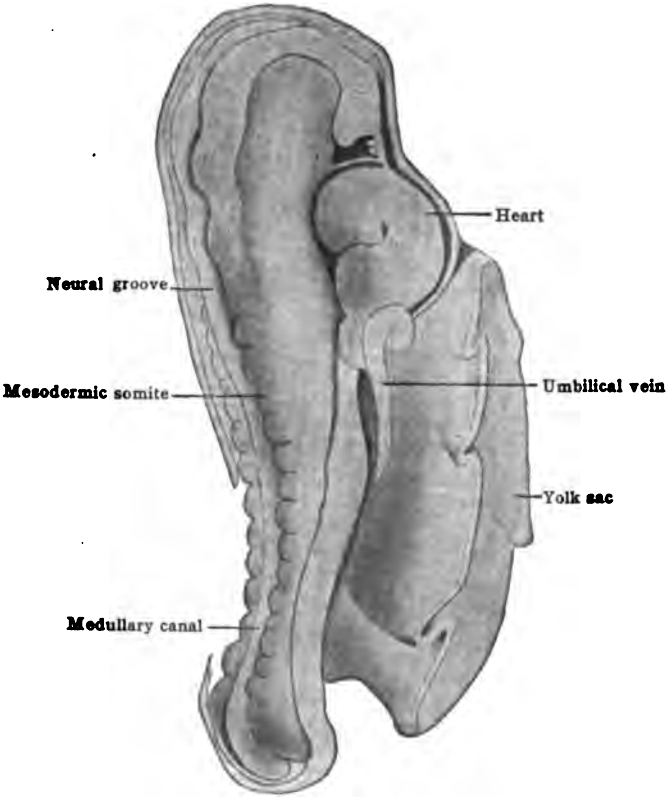
The closure begins, not at the anterior end (as might be expected from the general law of cranio-caudal development), but in the cervical region, extending forward into the brain region, and backward along the spinal cord. Thus the extreme ends (anterior and posterior neuropores) are the last to close.

The precocious and energetic growth of the neural anlage is largely responsible for the ventral flexure of the embryonic body axis, especially in the head region, where the flexures of the brain are very conspicuous (figs. 22, 26).

With the closure of the neural tube dorsally and of the alimentary canal ventrally the human embryo assumes the typical vertebrate form. The cylindrical body wall now encloses two tubes (neural and enteric) with the longitudinal axis (notochord) between them (figs. 18, 24).

After the embryonic disc has been transformed into a tube, the body of the human embryo in cross section appears not circular but elongated dorso-ventrally. This is the typical form for vertebrates with horizontal body axis. In later fœtal stages, the body becomes more rounded in cross section, and finally, with the assumption of the erect posture in postnatal life, becomes decidedly flattened dorso-ventrally (figs. 20, 21).

FIG. 17.—A HUMAN EMBRYO 2.5 MM. IN LENGTH. (After Kollmann.)



Development of the mesoderm.—The mesodermic layer on each side of the notochord in the embryonic disc develops in two divisions. The medial (or dorsal) divisions form a series of hollow segments, the *somites* (figs. 16, 17, 18). The lateral (later ventral) divisions each split into an upper (outer) or somatic layer and a lower (inner) or visceral layer. When the embryonic disc becomes folded, the corresponding somatic and visceral layers unite ventrally and enclose between them the common *cœlom* or primitive body cavity (fig. 18).

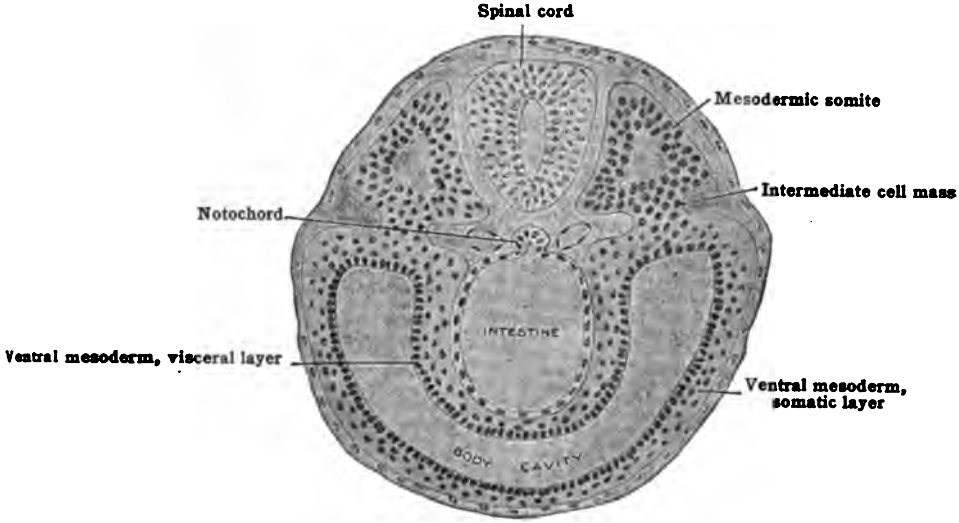
As previously noted, the mesoderm arises chiefly from the lateral portions of the 'head process.' A comparatively early stage before the appearance of the somites is shown in cross section in fig. 15. The somites appear first in the occipital region, and rapidly differentiate successively in the cranio-caudal direction (figs. 16, 17, 22). In embryos 7 or 8 mm. in length, about 40 somites may be distinguished, 3 to 5 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 5 or 6 coccygeal (in the rudimentary tail region).

The *cœlom* or body cavity is unsegmented. Two primitive pericardial cavities appear separate at first, but soon fuse and unite with the general cœlom. Later the general cœlom becomes secondarily divided into the permanent pericardial, pleural and peritoneal cavities.

The outer layer of the lateral mesodermic division forms the somatic or parietal layer of the peritoneum, etc. The inner layer forms the visceral or splanchnic layer, and develops not only the serous membrane, but also the muscular and connective tissue of the walls of the alimentary canal and its derivatives.

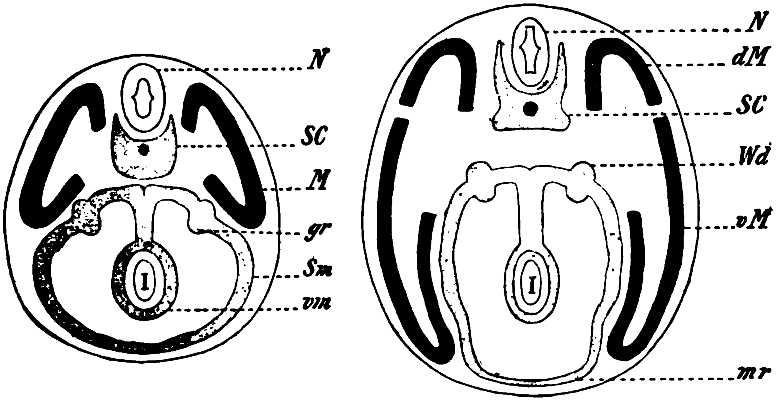
Development of the somites. Metamerism.—The appearance of the somites marks the beginning of *metamerism*, the arrangement of the body in successive segments or metameres. Each somite develops a primitive muscle segment, *myotome*, and a skeletal segment, *sclerotome* (figs. 18, 19). Moreover, the corresponding nerves and blood-vessels likewise assume a metameric arrangement. This metamerism persists (more or less modified) in the adult neck and trunk.

Fig. 18.—Diagram of a Cross Section of a Human Embryo.



The differentiation of the somites is illustrated by figs. 18 and 19. The medial wall of each somite forms the *sclerotome*. Its cells migrate to form the corresponding vertebra, rib, etc., as well as the *mesenchyme* forming the various connective tissues in this region. The remainder of the somite forms the *myotome*, from which the voluntary musculature of the trunk, the neck and (in part) the head is derived. The dorsal portions of the myotomes develop the muscle in the dorsal region of the trunk, while the ventral portions extend ventralward to form the musculature of the latero-ventral body walls (figs. 19, 20, 21, 23).

Fig. 19.—Diagrams Illustrating the History of the Mesoderm. *M*, myotome, *dM*, dorsal portion of myotome. *vM*, ventral portion of myotome. *SC*, sclerotome. *gr*, genital ridge. *Wd*, Wolffian duct. *Sm*, somatic layer of mesoderm. *vm*, visceral layer of mesoderm. *mr*, membrana reuniens. *I*, intestine. *N*, neural tube. (McMurrich.)

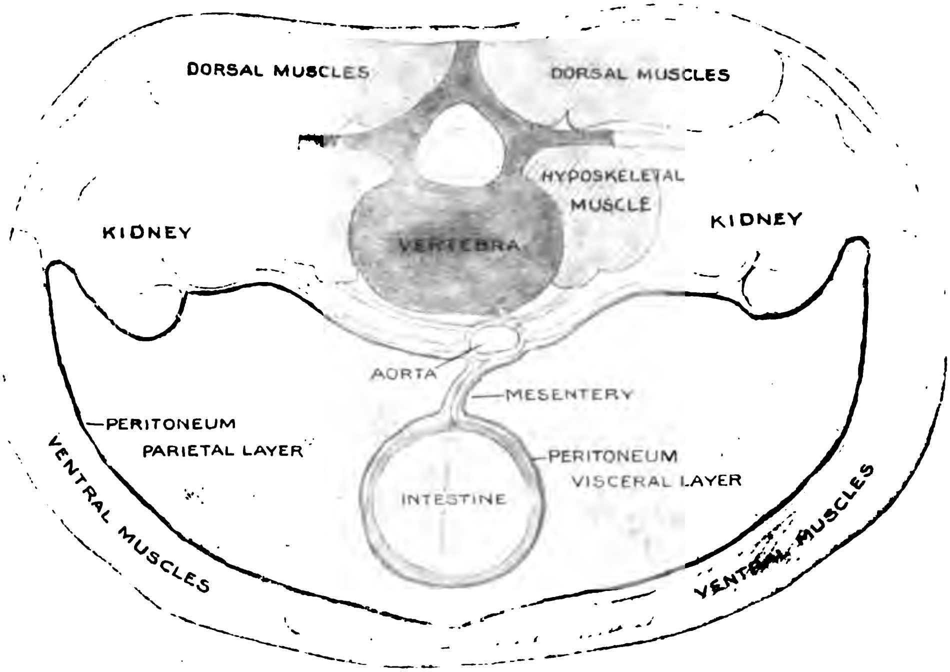


At the junction of the dorsal and ventral divisions of the mesoblast is a group of cells called the *intermediate cell mass*. This mass becomes segmented (corresponding to the somites) and each segment, or *nephrotome*, gives rise to a portion of the *mesonephros*, the provisional kidney. Other cells of the mass become *mesenchyme*, which is converted into blood-vessels, connective tissue, etc.

As development proceeds, the metamerism of the muscles and arteries becomes more or less obscured, but that of the vertebræ and nerves is fully retained even in the adult. In the case of the muscle plates, from which all the voluntary musculature of the trunk is derived, great modifications occur. Extensive fusion of successive plates occurs, the intervening connective tissue disappearing more or less completely; associated with this fusion there is longitudinal and tangential splitting of the somites to form individual muscles; and portions of some of the plates may wander far from their original position. But notwithstanding these complicated changes, indications of the primary metameric arrangement of the muscle plates are abundant, and even in the most extreme cases of modification the developmental history of a muscle can be determined by means of its nerve supply. For the fibres derived from each plate will usually retain, no matter what changes of independence or position they may undergo, the innervation by their originally corresponding segmental nerve; so that the occurence in the lumbar region of the body of muscle-fibres (the diaphragm) supplied by nerve-fibres from a cervical nerve is evidence that the muscle-fibres have been derived from a cervical mesodermic somite and have subsequently migrated to the position they finally occupy.

As regards the arteries, they arise primarily from a longitudinal stem, the aorta, in a strictly segmental manner, each metamere having distributed to it two pairs of arteries and a single median one (fig. 20). One pair of arteries supplies the body wall, and these retain very distinctly their original metameric arrangement; the other pair passes to the paired viscera, such as the lungs, kidneys, ovaries (or testes), so many of the pairs disappearing, however, that their metameric arrangement is not very evident in the adult. The unpaired vessels supply the digestive tract and its unpaired appendages, such as the liver and pancreas, and undergo great modifications, those of the lower thoracic and lumbar regions becoming reduced by fusion and degeneration to three main trunks.

FIG. 20.—DIAGRAM OF A TRANSVERSE SECTION THROUGH THE ABDOMINAL REGION.



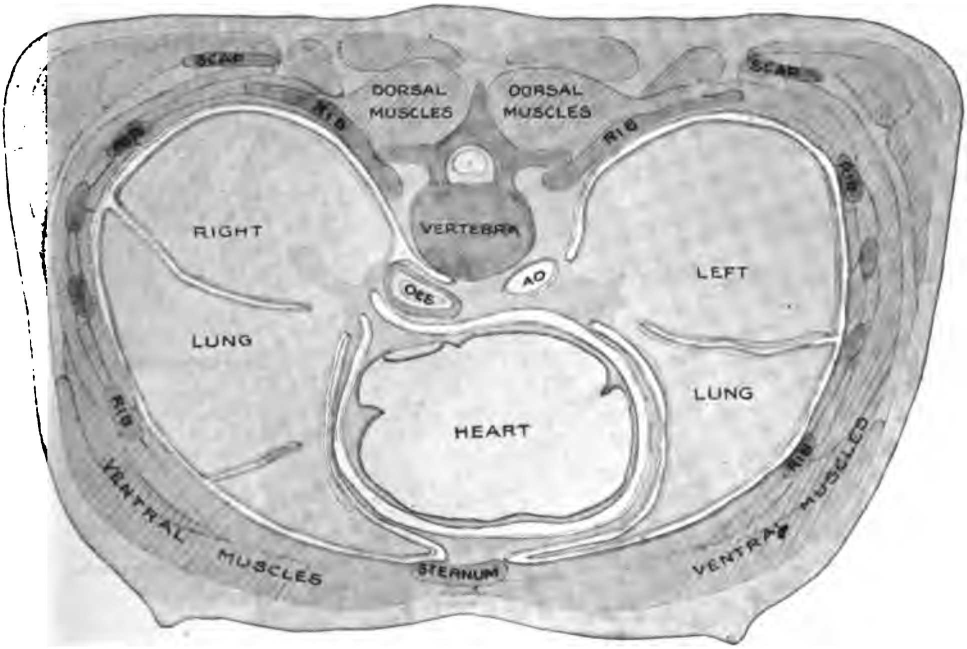
Branchiomerism.—Throughout the trunk and neck regions, then, a fundamental metameric plan underlies and determines the arrangement of many parts. In the head there is also evident a primary arrangement of the parts in succession; but this arrangement appears to be somewhat different from that of the trunk in that it involves the ventral instead of the dorsal mesoderm and is associated with the occurrence of branchial arches rather than with true mesodermic somites. It is consequently termed *branchiomerism.*

Not but that there are also indications of metamerism in the head, the muscles of the orbit, and the majority of the extrinsic muscles of the tongue, together with the nerves supplying these muscles, being apparently metameric structures, but the metamerism of this region of the body is largely overshadowed by the branchiomerism.

If an embryo of about the fifth week of development (fig. 22) be examined, there will be observed on the surface of the body in the pharyngeal region three or four linear depressions, and sections will show that similar and corresponding grooves also occur upon the inner surface of the pharyngeal wall. These are the *branchial grooves,* and since they are four in number (with a rudimentary fifth) in the human embryo, they mark off five *branchial* (*or visceral*) *arches,* the first of which lies between the oral depression and the first branchial groove, while the fifth is situated behind the fourth groove. These branchial arches are so named because they represent the arches which (excepting the first) support the gills (branchiæ) in the lower vertebrates, the grooves representing the branchial slits, even although they do not become perforated in the human embryo.

Each *branchiomere* consists of an axial skeletal structure, of muscles which act on this skeleton, of a nerve which supplies the muscles and the neighbouring integument and mucous membrane, and of an artery which carries blood to all these structures. The arches, however, do not in the human embryo retain their original branchial function, but undergo extensive modifications, becoming adapted to various functions and showing less in the adult of their originally simple arrangement than do the metameres. Nevertheless no matter what modifications the musculature of any arch may undergo, it will retain its original innervation and, to a large extent, its relations to the skeletal elements of its arch; and even the arteries in their distribution show clear indications of being arranged in correspondence to the various arches.

FIG. 21.—DIAGRAM OF A TRANSVERSE SECTION THROUGH THE THORACIC REGION. (The pleura is represented in blue and the pericardium in red.)



With respect to the fate of the various structures pertaining to each branchial arch, their general arrangement in the adult body may be stated in the following table:—

RELATIONS OF THE BRANCHIAL ARCHES IN THE ADULT

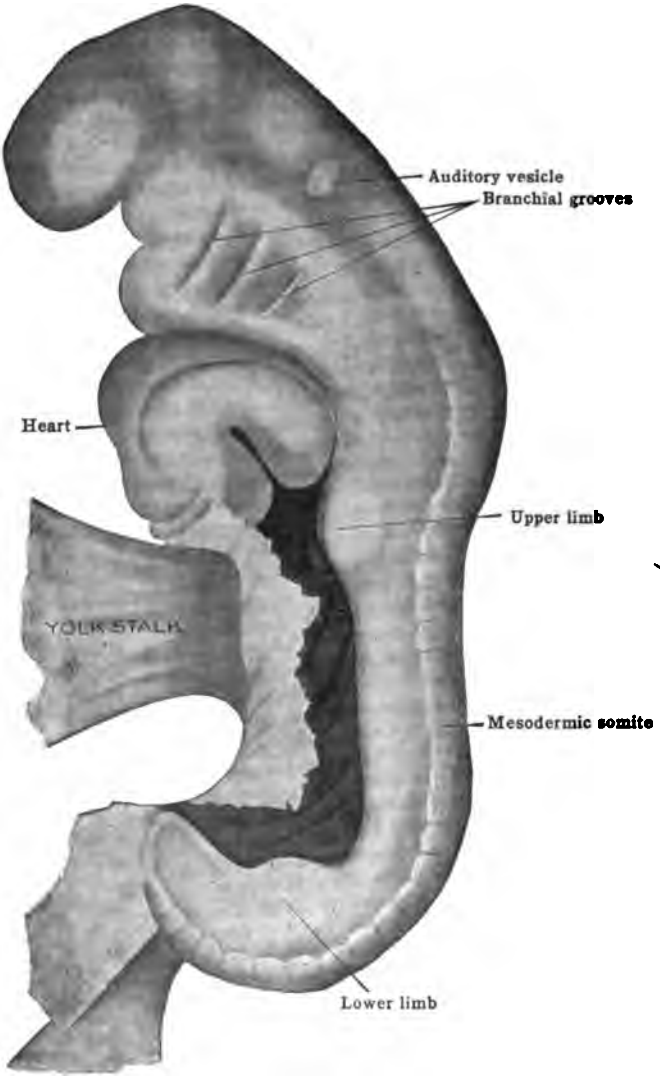
Arch	Skeleton	Muscles	Nerve
First arch.......	Mandible, malleus and incus.	Masticatory, mylohoid and digastric (ant.), tensor tympani.	Trigeminus.
Second arch.....	Hyoid (lesser cornu), styloid process and stapes.	Stylohyoid, digastric (post.), muscles of expression, stapedius.	Facialis.
Third arch......	Hyoid (greater cornu)...	Pharyngeal..................	Glossopharyngeus.
Fourth and fifth arches.	Thyreoid cartilage......	Pharyngeal and laryngeal.....	Vagus.

Branchial grooves.—Of the *external branchial grooves,* the first (lying between mandibular and hyoid arches) becomes deepened to form the *external auditory meatus,* the margins becoming elevated to form the *auricle* (fig. 26). The region corresponding to the second, third and fourth external grooves becomes depressed, forming the *sinus cervicalis,* which soon closes up and disappears.

The *internal branchial grooves* or pouches communicate with the pharyngeal cavity and are lined with endoderm. The first internal groove becomes transformed into the *auditory* (Eustachian) *tube*, *tympanic cavity*, etc. The second internal groove persists in part as the fossa of the *palatine tonsil*. The third and fourth grooves are probably represented in part by the *vallecula* and *recessus piriformis*, detached portions of their lining endoderm giving rise to the *thymus*, *parathyreoid glands*, etc. The rudimentary fifth groove is said to give rise to the *ultimobranchial body*, a structure of uncertain significance (fig. 27).

Development of the face.—The facial region is at first relatively small. It includes the sense organs (eye, ear, nose) and mouth region. Some of the more important developmental features may be briefly mentioned. In an embryo of the sixth week (fig. 28) the wide mouth aperture is seen to be bounded below (posteriorly) by the lower (*mandibular*) portion of the first arch, laterally by the upper (*maxillary*) process of the first arch. Above it is bounded by a median plate, the *nasal process*, which on either side forms a protuberance, the *globular process*. Lateral to the globular process is a rounded depression, the *nasal pit*. The maxillary process extends forward and fuses with the globular process to form the upper jaw region (failure to unite resulting in the malformation known as 'hare-lip'). The nose is at first broad, due to the width of the nasal process, which later becomes the nasal septum (fig. 29). The nasal pits deepen and later acquire openings into the primitive mouth cavity.

FIG. 22.—HUMAN EMBRYO OF 4.2 MM., SHOWING THREE BRANCHIAL GROOVES. (After His.)



The viscera.—The structures so far considered belong, for the most part, to the body wall; it remains to consider the general plan of arrangement of the viscera. It has been pointed out that the body may be regarded as a cylinder enclosing two tubes, one of which constitutes the central nervous system and the other the digestive tract. The latter may be regarded as being primarily a straight tube traversing lengthwise the body cavity enclosed by the body wall (figs. 18, 20). The layers of both the visceral and somatic plates which immediately enclose the body cavity become transformed into a characteristic pleuro-peritoneal membrane. Near the mid-dorsal line, a vertical double plate of peritoneum extends ventrally connecting the somatic (parietal) and visceral layers of peritoneum, and constituting what is termed the *mesentery* (fig. 20).

Fig. 23.—Sagittal Section Showing the Primitive Pericardial and Cœlomic Communication, Septum Transversum, Liver, etc., in a Human Embryo of 3 mm. (After Kollmann, from a model by His.)

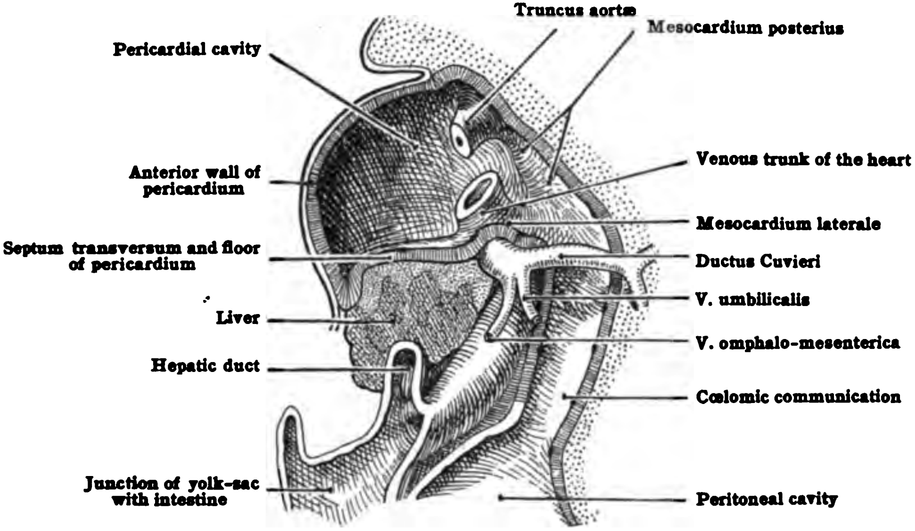
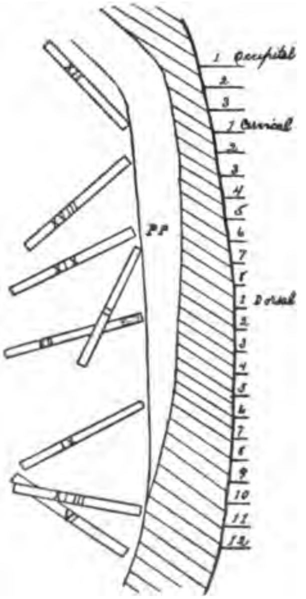


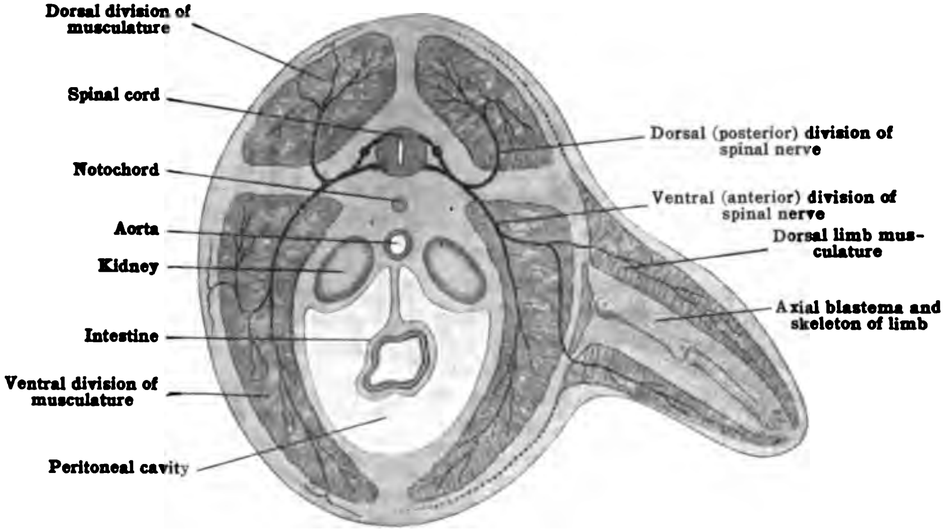
Fig. 24.—Diagram Illustrating the Recession of the Diaphragm (Septum Transversum) in the Human Embryo. On the right are indicated the vertebral levels; on the left, the position of the septum transversum in a series of embryos from 2 mm. (XII) to 24 mm. (VI) in length. *pp*, pleuro-peritoneal cavity. (Mall.)



As development proceeds the digestive tract grows in length more rapidly than the cavity which contains it, and so gradually becomes thrown into numerous coils in the abdominal region, these changes leading to numerous modifications of the original arrangement of the mesentery. These will be described later on in the section on the digestive system. Several outgrowths also arise from the primitive digestive tract, to form important organs, such as the lungs, the liver, the pancreas and the urinary bladder; and, with the exception of the bladder, each of these becomes completely invested by primitive peritoneum. In the case of the liver this original condition is practically retained, but the investment of the pancreas later becomes a partial one on account of the modifications which ensue in the mesentery. The bladder has only a portion of its surface in contact with the peritoneum, but the investment of the lungs remains complete, each lung, indeed, appropriating to itself the entire visceral layer of its half of the thorax, with the exception of a small ventral portion which forms the investment of the heart. Furthermore, the cavities which surround each of the three organs named, the two lungs and the heart, become completely separated from one another; and since each investment consists of a visceral and a parietal layer, each of the organs is enclosed within a double-walled sac, which in the case of each lung forms its *pleura*, while that of the heart is known as the *pericardium*. The spaces which occur within the thorax between the pleuræ of the two sides are known as the *mediastina*, which include the heart, œsophagus, etc. (fig 21).

In addition to the viscera mentioned there are some organs, such as the spleen and genito-urinary organs, which are developments of the mesoderm, the spleen arising in the mesentery which passes to the stomach and the genito-urinary organs primarily from the intermediate cell mass. The morphogeny of these structures and also of the vascular system, nervous system, and sense organs will be considered later in connection with their structure.

FIG. 25.—DIAGRAM OF A CROSS SECTION OF THE EMBRYONIC BODY AND LIMB. (McMurrich, after Kollman.)


Dorsal division of musculature
Spinal cord
Notochord
Aorta
Kidney
Intestine
Ventral division of musculature
Peritoneal cavity
Dorsal (posterior) division of spinal nerve
Ventral (anterior) division of spinal nerve
Dorsal limb musculature
Axial blastema and skeleton of limb

Recession of the diaphragm and heart.—In the early stages of development the heart is situated far forward, in what will eventually be the pharyngeal region (figs. 12, 17). Just behind (caudal to) the heart, between it and the yolk-sac, is a plate of connective tissue, the *septum transversum*, which serves for the passage of large veins from the body wall to the heart (figs. 17, 23). This septum together with certain accessory structures eventually gives rise to the diaphragm, which becomes a complete partition separating the thoracic and abdominal portions of the body cavity.

The diaphragm and heart are therefore originally situated far above (cranial to) their final position and recede in the course of development, producing an elongation of the vessels and nerves associated with them and forcing downward such organs as the stomach and liver (fig. 24). The chief factor in this displacement is probably the ventral head flexion and the precocious growth and expansion of the organs in the head region. The effects of this recession are especially noticeable in the nerves, these passing to the various organs concerned arising from a much higher level than that occupied by the organs. The nerve to the diaphragm, for instance, comes from the fourth cervical segment, those passing to the cardiac and pulmonary plexuses from the cervical region, and those to the plexus in relation with the stomach, liver and adjacent organs from the thoracic region. The blood-vessels, however, may shift their origins from the main trunks by successive anastomotic roots, so that in general they keep pace with the viscera in the migration caudalward.

The limbs.—Each limb at its first appearance (fig. 22) is a flat, plate-like outgrowth from the side of the body, and consists of an axial mass (blastema) of mesodermic tissue from which the limb skeleton will develop, and, surrounding this, a layer, also of mesodermic tissue, from which the muscles and blood-vessels will arise. It is as yet uncertain whether the muscle blastema is derived from the myotomes (as in lower vertebrates) or whether it develops from the mesenchyme

FIG. 26.—LATERAL VIEW OF A HUMAN EMBRYO 18 MM. LONG, SHOWING THE DEVELOPMENT OF THE EXTREMITIES. M, mandibular arch.

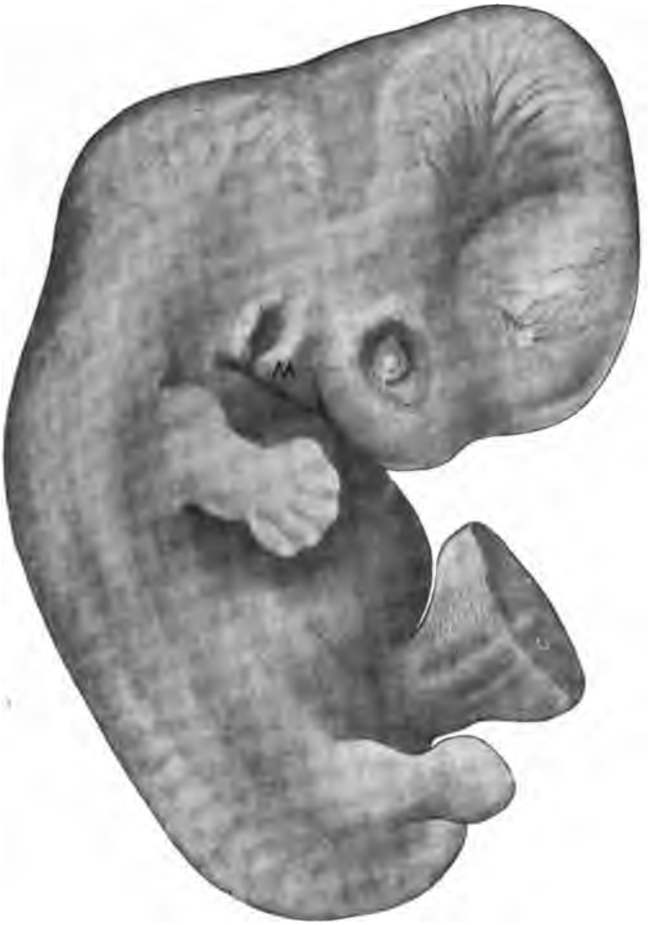
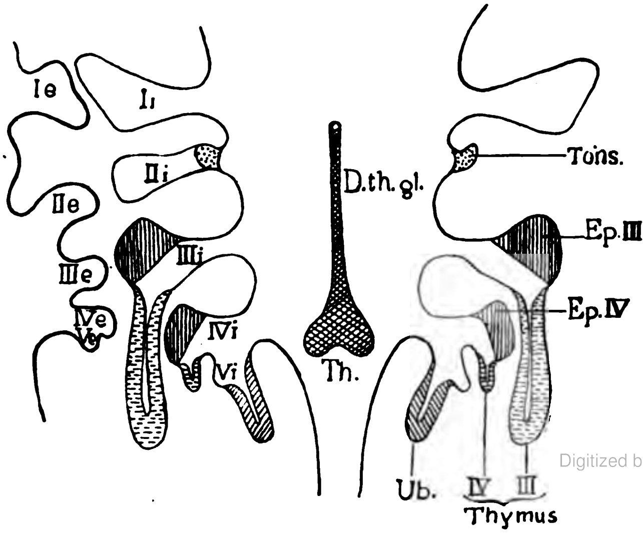


FIG. 27.—DIAGRAM TO SHOW THE DERIVATIVES OF THE BRANCHIAL CLEFTS. I*e*, II*e*, III*e*, IV*e*, V*e*, external branchial grooves. I*i*, II*i*, III*i*, IV*i*, V*i*, internal branchial grooves. Tons., palatine tonsil. *Ep III*, *Ep* IV, epithelial bodies. *Ub*, ultimobranchial body. *Th.*, thyreoid gland. *D.th. gl.*, ductus thyreoglossus. (Modified rom Keibel and Mall.)



As the muscles become differentiated, nerves grow to them from a definite number of spinal segments (fig. 25).

At first each limb plate is so placed that one of its surfaces looks dorsally and the other ventrally, and one border (that corresponding to the thumb or great toe) is anterior (i. e., cranial) and the other posterior (caudal). Later, however, each limb becomes bent caudally through about ninety degrees, so that the limbs whose long axes were at first at right angles to the long axis of the body come to lie parallel to that axis. In addition there occurs a rotation of each fore-limb in such a manner that the thumb turns latero-dorsally, while in the lower limb the direction of the movement is exactly the opposite, the great toe turning ventro-medially. As a result there is an apparent reversal of the surfaces in the two limbs, the flexor muscles of the arm reaching on the surface which is directed anteriorly, while in the lower limb the corresponding muscles occupy the posterior surface. The dorsum of the foot and the great toe side correspond respectively to the back and thumb side of the hand, the tibia corresponds to the radius and the fibula to the ulna. The limb anlage soon becomes divided into three primary segments. The distal segment (hand or foot) is a flattened rounded disc, in which the digits soon appear (fig. 26). The proximal portion forms the forearm or leg and the arm or thigh. In general, the extremities follow the law of cranio-caudal and dorso-ventral (proximo-distal) development.

Fig. 28A.—Face of Human Embryo of about 8 mm. (His.)

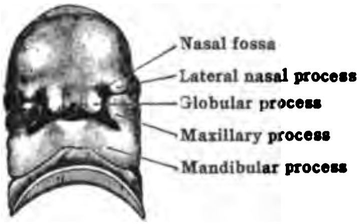
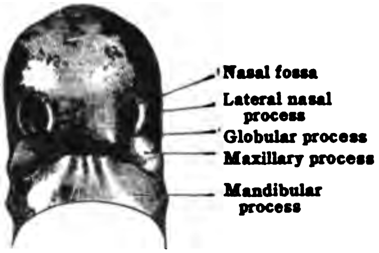


Fig. 28B.—Face of Human Embryo at Stage Slightly Later than 28A. (After Kallius.)



Prenatal Growth in Length and Weight

Age in lunar months	Crown-rump or sitting height (Mall), cm.	Crown-heel or standing height (Mall), cm.	Weight at end of month, grams	Ratio of increase to weight at beginning of month
0	(diameter of ovum =0.2 mm.)	....	(Ovum = 0.000004 g.)	....
I	0.25	0.25	0.004	999.0
II	2.5	3.0	2.0	499.0
III	6.8	9.8	24.0	11.0
IV	12.1	18.0	120.0	4.0
V	16.7	25.0	330.0	1.75
VI	21.0	31.5	600.0	0.82
VII	24.5	37.1	1000.0	0.67
VIII	28.4	42.5	1600.0	0.60
IX	31.6	47.0	2400.0	0.50
*X	33.6	50.0	3200.0	0.33

Prenatal growth.—The prenatal growth of the human body in length and weight is indicated in the preceding table. According to Hasse, the age of the fœtus may be estimated from its total length as follows. Before the fifth month, the square of the age in (lunar) months gives the length in centimetres. After this, the age in months multiplied by five gives the length. This gives approximate results, except for the first month.

While the growth in *absolute* weight increases from month to month, it is important to note that the real (relative) growth rate rapidly diminishes. The ovum increases in weight during the first month about 1000 times, or 100,000 per cent. (not including the extra-embryonic structures). This rate diminishes rapidly, however, so that the increase during the last fœtal month is only about 33 per cent.

The continuation of growth in length and weight during the postnatal period is shown in the following chart (fig. 30).

The following chart is based upon data from Camerer (1–5 yrs.), Porter (6–17 yrs.), and Roberts (18–20 yrs.), showing the average postnatal growth in height and weight by sexes. The average height at birth is about 50 cm. (20 inches); weight, about 3200 g. (7 pounds). The male is slightly heavier and taller than the female, except during the acceleration at the period

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* 270 days (Mall).

FIG. 29.—FACE OF A HUMAN EMBRYO AFTER COMPLETION OF THE UPPER JAW. (McMurrich from His.)

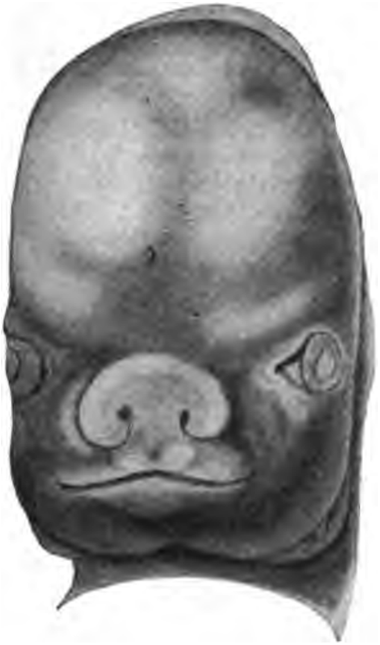
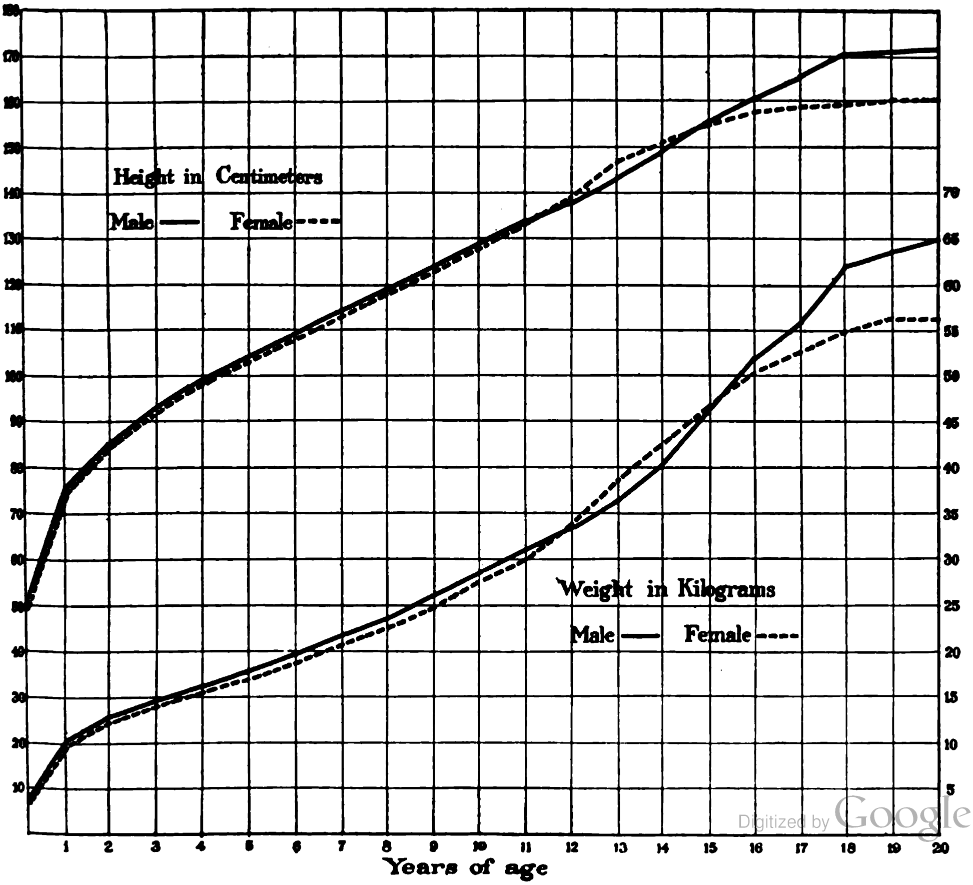


FIG. 30.—CHART SHOWING AVERAGE POSTNATAL GROWTH IN HEIGHT AND WEIGHT.



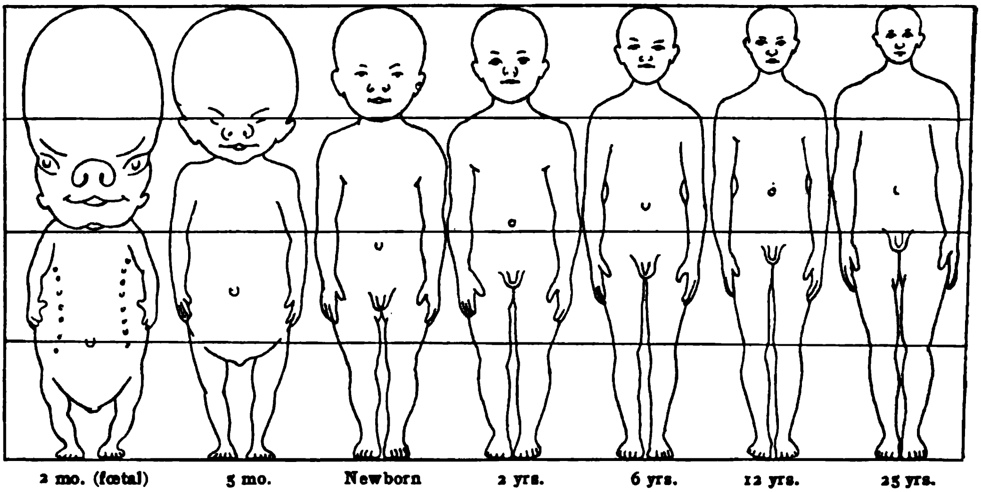
of puberty. Puberty occurs earlier in the female, so that between the ages of 12 and 15 the girls exceed the boys in average height and weight. With the exception of this period of acceleration, the (relative) growth rate in general diminishes steadily from birth, and has practically ceased at 20 years. The average height at this time is about 160 cm. (5 ft., 3 in.) in the female, and 170 cm. (5 ft., 7 in.) in the male; average weight, about 56 kilograms (126 lbs.) in the female, and 65 kilograms (146 lbs.) in the male. Under favourable conditions, growth in height may continue slowly up to about 25 years, and in weight even longer; but in old age there is a slight decrease in both height and weight.

The following measurements (from Holt, "Diseases of Infancy and Childhood" may be taken as a normal average standard of growth during the first three years. The weights are taken without clothing. The height is taken by placing the baby on a perfectly flat surface like a table, and having some one hold the child's knee down so that he lies out straight, then taking a tape-measure and measuring from the top of his head to the bottom of his foot, holding the tape line absolutely straight. The chest is measured by means of a tape line passed directly over the nipples around the child's body and midway between full inspiration and full expiration. The head measurement is taken directly around the circumference of the head, over the forehead and occipital bone.

		Weight, pounds	Height, inches	Chest, inches	Head, inches
Birth........	Boys	7.55	20.6	13.4	13.9
	Girls	7.16	20.5	13.0	13.5
6 months....	Boys	16.0	25.4	16.5	17.0
	Girls	15.5	25.0	16.1	16.6
12 months...	Boys	20.5	29.0	18.0	18.0
	Girls	19.8	28.7	17.4	17.6
18 months...	Boys	22.8	30.0	18.5	18.5
	Girls	22.0	29.7	18.0	18.0
2 Years.....	Boys	26.5	32.5	19.0	18.9
	Girls	25.5	32.5	18.5	18.6
3 Years.....	Boys	31.2	35.0	20.1	19.3
	Girls	30.0	35.0	19.8	19.0

Relative growth of the parts.—The growth of the body is not uniform in the various parts, and changes in proportions therefore occur during development, as shown in fig. 31. It will be noted that the changes are in accordance with the law of developmental direction previously explained, the growth impulse passing along the body in a cranio-caudal direction.

Fig. 31.—Figures Illustrating the Changes in Proportion During Prenatal and Postnatal Growth. (Stratz.)


2 mo. (fœtal)
5 mo.
Newborn
2 yrs.
6 yrs.
12 yrs.
25 yrs.

The *head* is therefore largest in the earlier stages, forming about half the body, decreasing to 25 per cent. in the newborn, and to 7 or 8 per cent. of the body in the adult. The *upper limbs* increase to about 10 per cent. of the body at birth, maintaining thereafter about the same relative size. The *trunk* as a whole remains of about the same relative size (about 45 per cent.), although the thoracic portion reaches its maximum in the earlier stages, and the pelvic portion not until adult life. The *lower limbs*, like the pelvis, develop slowly, forming about 20 per cent. of the body at birth and reaching 35 per cent. in the adult.

Relative growth of the systems.—There is also a marked difference in the relative growth of the various systems. Data for the *skin* and *skeleton* are somewhat scanty and unsatisfactory. The *musculature*, however, is relatively small in the embryo, increasing to about 25 per cent. of the body in the newborn, and to 40 or 45 per cent. in the adult. The visceral group (including brain and spinal cord), on the other hand, is relatively largest in the early embryo, decreasing from about 35 per cent. of the body to about 24 per cent. in the newborn and to about 10 per cent. in the adult.

Relative growth of the organs.—While in general, the individual organs follow the course of relative growth of the visceral group, each organ has its own characteristic course of growth. As a rule, after its appearance in the embryo, each organ increases more or less rapidly to its maximum relative size, after which, although increasing in *absolute* size, it decreases in *relative* size through subsequent prenatal and postnatal life up to the adult.

Thus the *brain* in the embryo of the second month forms more than 20 per cent. of the body, but steadily declines to about 13 or 14 per cent. in the newborn, and about 2 per cent. in the adult. The *spinal cord* and *eyeballs* have a similar course of growth. The *heart* declines from about 5 per cent. of the body in the embryo of the second month to about .75 per cent. in the newborn and .46 per cent. in the adult. The *liver* decreases from a maximum of nearly 10 per cent. in the third month to 5 per cent. in the newborn and 2.7 per cent in the adult. The *suprarenal glands* decrease from about .46 per cent. of the body in the third month to .23 per cent. in the newborn and .01 per cent. in the adult. The *lungs* decrease from 3.3 per cent. in the fourth month to about 2 per cent. of the body at birth and 1 per cent. (bloodless weight) in the adult. The *kidneys* reach a maximum of about 1 per cent. of the body toward the end of the fœtal period, decreasing to about .46 per cent. in the adult. The *thymus*, *thyroid*, *spleen* and *alimentary canal* likewise reach their maximum slowly, being probably relatively largest about the time of birth. The *ovary* and *testis*, however, appear to be relatively largest during the prenatal period.

Variability.—It must be borne in mind that all statements concerning structure refer to the *average* or *norm*, and are always subject to variation. This is therefore a topic of importance to students of anatomy. Variations are classified as either *germinal* or *somatic*.

Germinal variations are due to fundamental differences in the germ plasm, and are transmitted by heredity. These include many of the characters whereby one individual differs from another. Variations according to sex are included under this class. Variations inherited from more or less remote ancestors are termed *atavistic* or *reversional*.

Somatic variations, or 'acquired characters,' are due to environmental influences, such as nutrition, temperature, shelter, disease, training, etc. While somatic variations may be very great, they do not affect the germ plasm and are not transmitted by heredity.

In many cases it is exceedingly difficult to distinguish germinal from somatic variations Size, for example, may be due to either or both. Moreover, somatic variations may be produced at any time after the fertilisation of the ovum. Very slight environmental changes are sometimes sufficient to produce a marked effect upon the delicately balanced mechanism of the developing embryo. Malformations and pathological conditions are thus often to be explained. As to the *extent* of variability, some characters are much more variable than others. Height, for example, is less variable than weight. Moreover, variability differs in the various parts and organs. In general, the head and head organs are less variable than the remainder of the body. The skeleton and musculature appear less variable than the integument and viscera.

Details concerning variations and methods for their measurement may be found in works on genetics and biometrical statistics.

References.—*Embryology:* Keibel and Mall, Human Embryology (2 vols.); Bryce, Quain's Anatomy, 11th ed., vol. 1; Minot, Laboratory Text-book of Embryology; McMurrich, Development of the Human Body. *Growth:* Minot, Age, Growth, and Death; Jackson, Amer. Jour. Anat., vol. 9; Anat. Record, vol. 3. *Heredity:* Davenport, Heredity and Eugenics; Walter, Genetics. *Biometry:* Davenport, Statistical Methods; Yule, Theory of Statistics.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 1, 1915

27. *Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats.* C. M. JACKSON, Institute of Anatomy, University of Minnesota, Minneapolis.

Twenty-one well-nourished adult rats were used, initial body weights varying from 182 to 367 grams. Fifteen rats were used for acute inanition, being allowed water but no food. They were killed after 6 to 12 days, the loss in body weight varying from 25 to 39 per cent (average loss, 36 per cent). Six rats were subjected to chronic inanition, being underfed so as to reduce the body weight slowly through a period of about five weeks, and were killed when the loss in body weight reached about 36 per cent. The results below stated apply in general to both acute and chronic inanition, unless otherwise specified. The published data of Donaldson, Hatai, Jackson and Lowrey are taken as normal for comparison. On account of the great variability of some organs and the relatively small number of observations, final conclusions are in some cases uncertain.

The head and fore-limbs lose relatively less than the body as a whole. Their relative (percentage) weight therefore increases. Of the systems —integument, skeleton, musculature, viscera and 'remainder'—the integument loses relatively nearly the same as the whole body, and therefore nearly maintains its original relative (percentage) weight. The same is true of the musculature, which however undergoes a somewhat greater loss in relative weight during chronic inanition. The visceral group, as a whole, undergoes little change in relative weight, decreasing slightly in acute inanition. The individual organs, however, vary greatly, as indicated below. The skeleton retains nearly its original absolute weight, and therefore increases greatly in relative weight. There is a corresponding decrease in the 'remainder,' due chiefly to loss of fat.

The individual viscera may be classified in three groups:

(1) The brain, spinal cord and eyeballs show little or no loss in absolute weight, compared with the normal at the initial body weight, hence their relative (percentage) weight has markedly increased with the diminution in body weight during inanition. The same is apparently true for the thyroid gland in acute inanition, but in chronic inanition there is apparently a loss, though relatively less than in the body as a whole. The suprarenal glands also apparently lose less in absolute weight than the body as a whole, hence their relative (percentage) weight increases.

(2) The heart, lungs, kidneys, testis, epididymis and hypophysis undergo nearly the same relative loss in weight as the body as a whole, therefore their relative (percentage) weight remains nearly the same. The thymus has already undergone age involution, and is therefore not materially affected by inanition.

(3) The liver and the alimentary canal (both empty and including contents) usually decrease in weight relatively more than the body as a whole. The spleen is exceedingly variable; in acute inanition it usually shows a marked decrease in relative weight (although averaging higher in chronic inanition).

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28. Changes in young albino rats held at constant body weight by underfeeding for various periods. C. M. JACKSON, Institute of Anatomy, University of Minnesota, Minneapolis.

Ten litters, including 65 rats, were used. Twenty-five rats were used as controls, including 11 at 3 weeks of age, 2 at 6 weeks, 6 at 10 weeks, 3 at 32 weeks, and 3 at 35 weeks. In addition, data previously gathered by observations upon several hundred normal rats were available for comparison. Forty rats were held at constant body weight by underfeeding for various periods, 8 rats from age of 3 weeks to age of 6 weeks; 3 rats from 3 weeks to 8 weeks; 22 rats from 3 weeks to 10 weeks; 1 rat from 3 weeks to 13 weeks; 1 rat from 3 weeks to 16 weeks; 2 rats from 6 weeks to 32 weeks; and 3 rats from 10 weeks to 35 weeks. On account of the great variability of some organs, the number of observations in some cases is insufficient for final conclusions.

As to body proportions, the relative weights of the head, trunk and extremities remain practically unchanged in young albino rats held at constant body weights.

Of the systems—integument, skeleton, musculature, viscera and 'remainder'—there is but little change in the weight of the musculature, visceral group (as a whole) and 'remainder.' There is, however, usually a marked decrease in the integument, counter-balanced by a marked increase in the skeleton. Thus under these conditions the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process, during which the musculature shows the greatest increase and the skeleton lags behind relatively.

The increase in the skeleton during constant body weight appears to involve the ligaments and cartilages as well as the bony skeleton. The skeletal growth tends to proceed along the lines of normal development, as indicated by changes in water content, by formation and union of epiphyses, and by relative elongation of the tail (compared with trunk length). The teeth also continue to develop normally (eruption of third molars).

The individual viscera may be classified in three groups.

(1) There is during the maintenance of constant body weight a well-marked increase in the weights of the spinal cord and eyeballs; usually also of the testis alimentary canal (both empty and including contents) and hypophysis.

(2) There is no marked change in the weights of the brain, heart, lungs, suprarenal glands, kidneys and epididymi. The liver is variable, with apparently a slight tendency to increase in the younger rats and to decrease in the older.

(3) There is always a marked decrease in the weight of the thymus ('hunger involution'); usually also of the spleen (earlier stages) and probably to a slight extent of the lungs and thyroid gland.

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EFFECTS OF ACUTE AND CHRONIC INANITION UPON THE RELATIVE WEIGHTS OF THE VARIOUS ORGANS AND SYSTEMS OF ADULT ALBINO RATS

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TWO FIGURES

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As emphasized by the author in a previous paper (Jackson '13), there is great need of a series of comprehensive growth norms, which would serve as a basis for experimental work and give a better insight into the growth process in animals. Through the investigations of Donaldson, Hatai, Jackson, Lowrey and others, considerable progress has been made toward the establishment of a growth norm for the albino rat. Since the normal growth process and the relative weights of the organs are frequently modified by malnutrition from various (sometimes unsuspected) causes, it is clearly of great importance to know the effects produced by inanition. In drawing conclusions from the results of any experiment upon animals, the possible effect of inanition should always be kept in mind. The present investigation upon this subject, which was begun at the University of Missouri, has been continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. The grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc.

MATERIAL AND METHODS

The material included 21 well-nourished adult albino rats (Mus Norvegicus albinus) of unknown age. Two of these (O 5 and O 6) were derived from the Missouri colony and 19 from a local Minnesota stock. Of the 21, 4 were females and 17 males. They had been fed chiefly upon grain (oats and corn) with occasional meat and vegetables, and were placed upon a bread and milk diet for some days before beginning the experiment. During the experiment they were kept in cages with wire-net bottoms, allowing the feces (which might otherwise be eaten) to drop through.

Fifteen of the rats were used for the acute inanition experiment. They were allowed plenty of water, but no food otherwise. They were weighed daily and the individual records kept. The initial body weights varied from 182 to 367 grams (table 3). They were killed after 6 to 12 (average 9) days,[1] the total loss in body weight varying from 25 to 39 per cent (average loss, about 33 per cent). This represents moderately severe, but not extreme inanition, as a well-nourished adult albino rat will probably lose 40 per cent of his body weight before death, if kept warm. When pushed to the extreme, however, the rat is likely to die unexpectedly, with undesirable post mortem changes. These might affect not only the structure but also the weight of the organs, through congestion and coagulation of blood, and should therefore be avoided. Three of the rats included (table 3, rats Nos. M 9, M 13, and S 28) were found dead, and in this case merely the weights of the head, extremities, integument, skeleton and musculature (which did not appear to be materially affected) are recorded.

---

[1] Bell ('11) found that the wild brown (Norway?) rat in captivity would live only 3 or 4 days, with loss of 25 or 30 per cent in body weight, when water is supplied but food withheld. Among animals in general, the older or larger are able to withstand inanition longer than the younger or smaller animals, probably on account of less rapid metabolism in the former.

In the rats subjected to acute inanition, the daily loss in body weight was fairly uniform in some individuals, but varied greatly in others. As might be expected, the loss is usually (though not invariably) greatest on the first day of the experiment, probably due to the reduction in contents of the stomach and intestines. In most cases (11 out of 15) the loss in body weight is greater during the first half than during the second half of the experiment, though the difference is usually slight. There is no constant relation between the percentage loss in body weight and either the initial body weight or the length of the inanition period. In general, the loss in body weight of albino rats during acute inanition is very similar in extent and variations to that found by Chossat ('43) for pigeons. Chossat demonstrated that the loss in body weight is greatest in the first third of the inanition period, slightly less in the last third, and considerably less in the middle third.

In order to compare the results of chronic inanition, 6 rats (all males) were fed upon a bread and milk diet gradually decreased in amount, so as to reduce the body weight about 1 per cent (of the initial weight) daily. Water *ad libitum* was also supplied. This was continued until the loss in body weight amounted to an average of about 36 per cent, which required about 5 weeks (table 3). The amount of food (entire wheat (Graham) bread soaked in whole milk), required for this purpose varied somewhat in individuals, but was approximately 10 per cent of the body weight daily. This is usually found insufficient for the maintenance of body weight in adult albino rats at ordinary room temperature. One rat (No. M 4) was found dead; the others were killed.

At the end of the experiment, in both acute and chronic inanition, the rats were killed by chloroform and the various organs and parts carefully dissected and weighed. The technique followed is that described by Jackson ('13) and Jackson and Lowrey ('12). The skeleton was first prepared as heretofore by carefully dissecting off the musculature, leaving the periosteum, ligaments and cartilages intact. This is recorded as 'ligamentous skeleton.' In most cases the periosteum and ligaments were then removed by maceration for about one hour in 1 per cent 'gold dust' solution, following which the bones were cleaned under water with forceps and camel's hair brush.[2] So far as possible, the cartilages, including the intervertebral disks, were left intact and included with the skeleton. The skeleton was then taken from the water (excess moisture removed by filter paper) and weighed as 'cartilaginous skeleton.' The skeleton was then dried for 30 days in a dust-proof case at ordinary room temperature. This was found insufficient to remove all moisture, so the skeletons were finally dried several days in an oven at about 90°C., until a constant weight was reached.

Portions of the various tissues and organs were preserved for histological study, which will be considered in a later paper.

As heretofore, in calculating the percentage weights, the net body weight (gross body weight less intestinal contents) is taken. The percentage weights of the organs are therefore slightly higher than they would be if calculated upon the basis of the gross body weight, but the difference is usually negligible, as the intestinal contents form only 2 or 3 per cent of the body weight during inanition.

---

[2] For this method of preparing the cartilaginous skeleton, I am indebted to Professor Donaldson of The Wistar Institute. He states (in a personal communication) that the fresh skeleton is apparently slightly reduced (about 3 per cent) in weight by the 'gold dust' treatment, but this difference is so small as to be practically negligible.

For purposes of comparison, the previous observations upon the normal weights of the various organs of the albino rat by Donaldson ('09), Hatai ('13), Jackson ('13) and Jackson and Lowrey ('12) were utilized. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of six steers, varying from very fat to thin, are cited through the courtesy of Professors Trowbridge and Moulton.

The averages given in table 3 are the arithmetical means of the corresponding individual observations in the acute and the chronic inanition series, respectively. In estimating the normals at corresponding initial body weights for comparison, however, merely the averages were used. That is, the absolute weight of each organ corresponding to the *average* body weight was estimated (from data already available for the normal rat), and the corresponding percentage weight calculated. This is not quite so accurate as it would have been if the corresponding normal for each individual had been estimated, and the mean of these taken for comparison with the averages in the acute and chronic inanition series. However, the difference is slight and apparently not sufficient in the present series to justify the more laborious method of making the individual estimates.

In view of the comparatively small number of observations, and the known variability, especially of some of the organs (Jackson '13), the conclusions reached in the present paper are by no means to be considered as final. It is believed, however, that they are sufficient to give an approximate idea of some of the more obvious and important changes in weight during inanition. As such they may be useful, even though limited in number, and may lead to further and more extensive investigations in the case of various individual organs. In general, the amount of variation found is sufficient to demand the exercise of caution in drawing conclusions from an insufficient number of observations, as is sometimes done in experimental work.

Although the literature on the subject of inanition is extremely large, comparatively few specific data directly bearing upon the question of the changes in the weight of the various organs are to be found. These are referred to later under the appropriate headings. Papers dealing merely with histological changes are usually not mentioned. Extensive references to the literature on inanition will be found in the articles by Morgulis ('11) and Mühlmann ('99).

LENGTH OF BODY AND TAIL

With reference to the body weight compared to the body (nose-anus) length at the end of the inanition period, the normal relations as determined by Donaldson ('09) were used for comparison. As might be expected, the body weight following inanition, both acute and chronic, is found to be lower than normal for corresponding trunk length, since the skeleton is known to be in general but slightly affected by inanition. The difference, however, is surprisingly small. It is a noteworthy fact that the *initial* body weight of the animals used in he experiments is far greater than that normal for the body length at the *end* of the inanition period. (The length cannot be accurately measured in the living animal without anesthetics at the *beginning* of the experiment.)

TABLE 1

	ACUTE INANITION SERIES: GRAMS	CHRONIC INANITION SERIES: GRAMS
Normal gross body weight (theoretical, Donaldson) corresponding to body length at end of inanition period, average (from individual calculations)	191	147
Actual gross body weight found at beginning of the inanition period, average (table 1)	255	214
Actual gross body weight found at end of the inanition period, average	170	136

Thus, as shown in table 1, the body length at the end of the inanition period corresponds to a body weight much nearer to the final than to the initial weight of the animals subjected to inanition.

There are two possible explanations for this surprisingly close approach to the normal in the body weight after inanition. It is possible that the rats at the beginning of the experiment were somewhat too heavy for the normal at corresponding body length. This might account for the result, even on the assumption that the body length has remained constant during the period of inanition. On the other hand, it is more probable that there has been an actual slight decrease in the trunk length during inanition (probably due to shrinkage of the intervertebral disks), which would equally well account for the facts observed. Individual variations must also be kept in mind, as well as the possibility that the normal ratio of body weight to body length in the strain of rats used may differ somewhat from the normal, as determined by Donaldson.

The theory of a decrease in the trunk length during inanition is strengthened by the apparently changed ratio of tail length to body length. In a separate paper (Jackson '15) I have shown that the normal ratio of the tail length to body length in the albino rat increases from an average of about 0.36 in the newborn to 0.88 at 6 weeks (body weight 50 grams), decreasing slowly to about 0.85 at a body weight of 200 grams and to about 0.80 at 300 grams. (The lower ratio found in the heavier rats is partly due to the absence of females, in which the tail averages relatively longer than in the males.) Thus it appears that in rats with body weights corresponding to the initial weights in the inanition series, the normal average ratio of tail length to body length should be between 0.80 and 0.85. It is impracticable to make the actual measurements on the living animals, although this might be done by the use of anesthetics. The data given in table 3 show that at the end of the inanition period, however, the average ratio in the acute inanition series is 0.93, and in the chronic inanition series, 0.97. Thus it appears that inanition in adult albino rats tends to produce relatively long-tailed individuals, due probably to a shrinkage in the trunk length.

Hatai ('08) reached the opposite conclusion, viz., that underfeeding produces *short-tailed* individuals; but his observations were upon younger, growing rats, in which the conditions might be somewhat different. Morgulis ('11) in the salamander, Diemyctylus, likewise found a greater shrinkage in the tail during inanition, while Harms ('09) found the converse to be true in

Triton. This question is discussed more fully in the paper above referred to (Jackson '15), the conclusion being that inanition in young rats also tends to produce relatively long-tailed individuals.

HEAD

The head (fig. 1; table 3) in acute inanition averages about 11.2 per cent of the body weight, varying from 10 per cent in the larger rats to 13.9 per cent in the smaller. Normally (Jackson '13; Jackson and Lowrey '12) in rats corresponding to the body weight at the beginning of the experiment, the head should range from about 8 per cent in the larger rats to 10 per cent in the smaller, the average for the group being about 9 per cent. Thus we may assume that during the period of acute inanition the head has increased from an average of about 9 per cent to about 11.2 per cent of the body. This is an increase of about one-fourth in the relative size of the head.

Since the whole body has lost an average of one-third in absolute weight, it might appear at first glance that the head has remained nearly constant in absolute weight. This, however, is not true. If the body weight decreased one-third while the head remained constant in absolute weight, the *relative* (percentage) weight of the head would increase in the ratio of 2:3 or from 9 to 13½ per cent. A study of the absolute weights of the head, compared with the normal, at the beginning of the experiment shows that the head has actually lost weight, but in much smaller proportion than the body as a whole. This is what is to be expected since, as will be shown later, the brain, eyeballs and skeleton in general lose but little or none in absolute weight during inanition; while the loss in fat (some of which is on the head) is great, and the loss in the integument and musculature is in nearly the same proportion as in the body as a whole.

The head of the 6 rats subjected to chronic inanition averages 11.4 per cent of the body weight, slightly higher than in acute inanition. The rats used in chronic inanition averaged smaller in body weight (table 3), and the normal relative initial weight of their heads should therefore be slightly higher, about 10 per cent (instead of 9 per cent, as in the acute inanition group).

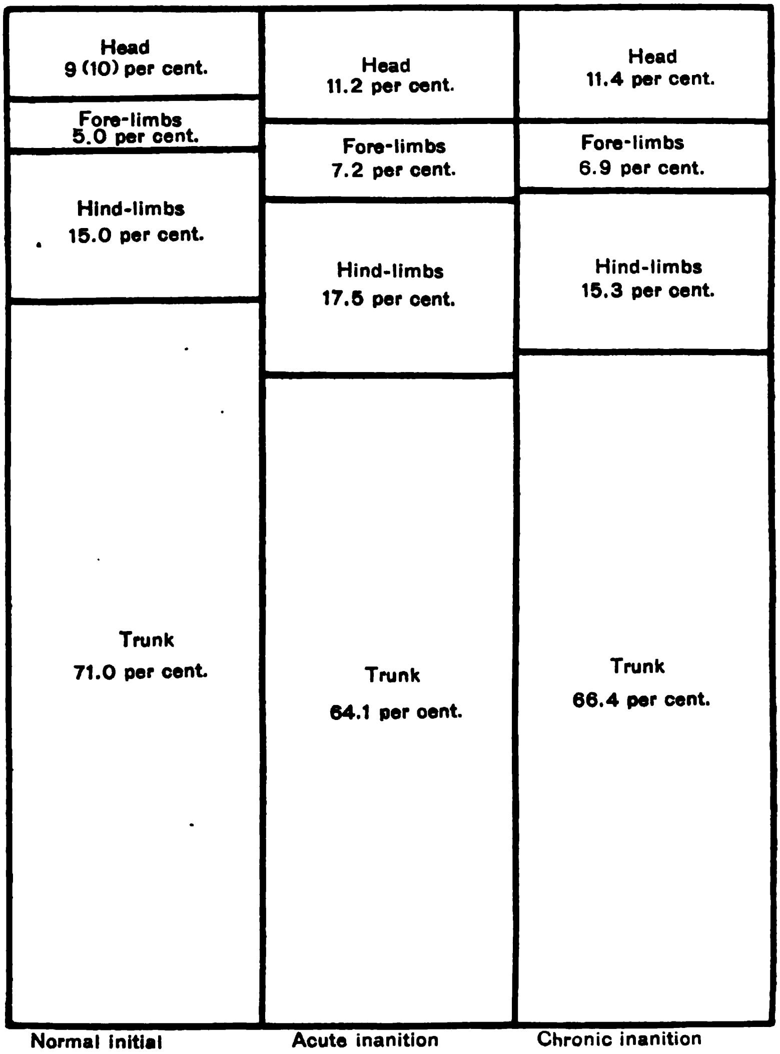

Fig. 1 Diagram representing the relative (percentage) weights of the head, trunk and extremities of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

EXTREMITIES AND TRUNK

The extremities were separated from the trunk at the shoulder and hip-joints, respectively. From figure 1 and table 3 it is seen that the *fore limbs* formed an average of 7.2 per cent of the body in the acute inanition series, and 6.9 per cent in the chronic inanition series. According to Jackson and Lowrey ('12), in the normal adult rat the fore limbs form about 5 per cent of the body. Consequently it would appear that there has been a relative increase in the weight of the fore limbs during inanition, especially during acute inanition. This increase is greater than would be expected, since (as will appear later) the skeleton is the only important constituent of the limbs which increases in relative weight during inanition, the integument and musculature remaining relatively constant. The conclusion as to the forelimbs should be regarded as uncertain, however, because of: (1) the difficulty in separating the limbs (especially the skin) in a uniform manner; (2) the comparatively small number of observations upon which both the normal and the experimental average is based; (3) the apparent lack of agreement between the results for the entire fore limbs and for their components, skin, skeleton and musculature. It is possible, on the other hand, that the losses for skin, skeleton and musculature are not uniform in all parts of the body. Chossat ('13) has shown, for example, that the great pectoral muscles in pigeons lose relatively much more than the remainder of the musculature during inanition.

The *hind limbs* (fig. 1; table 3) in the normal adult form about 15 per cent of the body (Jackson and Lowrey). As might be expected, this is slightly increased, to 17.5 per cent, in the acute inanition series, probably on account of the relatively heavier skeleton. In the chronic inanition series, the hind limbs average 15.3 per cent, or about the same as normal. This may be explained as due to the relatively greater loss in the integument and musculature during chronic inanition (as will be shown later), this loss tending to counterbalance the relative increase in the skeleton.

The *trunk* (fig. 1; table 3) is measured by substracting the weight of the head and extremities from the net body weight.

It forms normally about 0.71 per cent of the adult body (Jackson and Lowrey). During inanition, the trunk becomes relatively smaller, averaging 64.6 per cent in the acute inanition series (64.1 per cent, corresponding to the average of 11.2 per cent for the head in a larger series), and 66.4 per cent in the chronic inanition series. The relative decrease in the trunk of course counterbalances the relative increase in the head and extremities.

INTEGUMENT

As shown in figure 2 and table 3, the integument (which includes the skin and appendages, hair and claws) is fairly uniform in its relative weight, averaging 18.9 per cent in acute inanition (or 19.1 per cent in those cases in which the viscera were weighed), and 17.8 per cent in chronic inanition. The average integument for normal adults (fig. 2) corresponding to the initial size of these rats forms about 18 per cent of the body weight (Jackson and Lowrey '12). It is therefore evident that during both acute and chronic inanition in adult albino rats the loss in weight of the integument is nearly proportional to that of the body as a whole, so the relative (percentage) weight remains almost the same.

In absolute weight, the integument has apparently decreased from about 45.9 grams, the normal at the average initial body weight in the acute inanition series (255 grams), to an average of 31.6 grams, as shown in table 3. This would correspond to a loss of 31.2 per cent in the weight of the integument. In the chronic inanition series, the corresponding decrease is from 38.5 to 23.7 grams, a loss of 38.4 per cent. Apparently, therefore, the loss in the weight of the integument is relatively slightly greater during chronic than during acute inanition.

The data found in the literature concerning the relative loss of the integument during inanition are not in agreement. In the dog, Aron ('11) states that the skin loses relatively more than the muscles (therefore, more than the body as a whole); Voit ('05 b) cites data showing a relative increase in the (fat-free) skin; while Falck's ('54) data show the skin relatively unchanged in weight. In the rabbit and cat, observations by Pfeiffer ('87), Voit ('66)

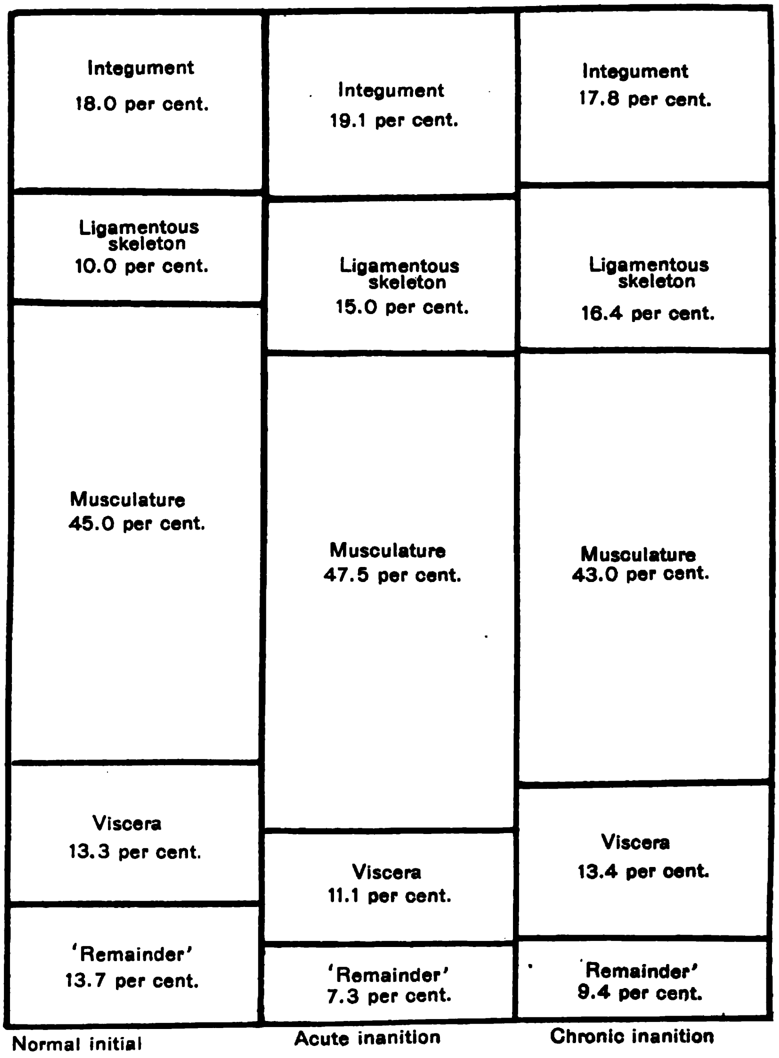

Fig 2 Diagram representing the relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

and Sedlmair ('99) show a relative increase in the skin during inanition. Much of the variation is doubtless due to the differences in the relative amount of fat present in the integument of the normal animal. The subcutaneous fat is not abundant in the rat. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of steers show that the percentage of the hide increases from 5.5 per cent in a very fat animal to 8.5 per cent of the body in a thin animal.

SKELETON

The ligamentous skeleton (table 3; fig. 2) forms an average of 15.3 per cent of the body after acute inanition (15.0 per cent in the series in which the viscera were weighed), and 16.4 per cent after chronic inanition. Since the average for the normal rat corresponding to the initial body weight of the animals used is only about 10 per cent of the body weight (Jackson and Lowrey '12), it is evident that while the body weight has decreased one-third during the period of inanition, the ligamentous skeleton has decreased little or none in absolute weight. If the normal skeleton formed 10 per cent of the body, and remained constant while the body lost one-third in weight, the *relative* weight of the skeleton would be increased one-half, or to 15.0 per cent. This corresponds with the observations as closely as could be expected.

In terms of absolute weight, assuming that the skeleton formed 10 per cent of the normal initial weight, the skeleton apparently decreased from 25.5 to 25.4 grams in the acute inanition series (loss of 0.4 per cent); and *increased* from 21.4 to 21.8 grams in the chronic inanition series (*gain* of 1.8 per cent). No great stress can be laid upon the accuracy of these figures, however, on account of the small number of observations, and the variability and uncertainty as to the normal weight.

The cartilaginous skeleton, including the bones and cartilages after removal of the ligaments by maceration (as previously described), forms an average of 10.9 per cent of the body in seven cases of acute inanition, and 12.4 per cent in the chronic inanition series (table 3). No published observations upon the normal cartilaginous skeleton are available for comparison. Professor Donaldson of The Wistar Institute, however, has very kindly sent me a series of observations upon the weight of the cartilaginous skeleton in normal albino rats. The average, in 10 cases with body weight varying from 194 to 426 grams, forms about 6.7 per cent of the (gross) body weight. In this series, however, I understand that the intervertebral cartilages were not preserved, so this figure is probably somewhat too low for use as a normal to be directly compared with my observations upon the cartilaginous skeleton in inanition. The normal adult cartilaginous skeleton, including the intervertebral cartilages, would *probably* form about 7 per cent (or slightly more) of the net body weight.

The dried cartilaginous skeleton (table 3) formed an average of 5.48 per cent of the net body weight in 7 cases of acute inanition, and 6.03 per cent in the 6 chronic cases. Thus it is evident that in each series the cartilaginous skeleton is composed of approximately half dry substances and half water. In the acute inanition series, the dry substance in the cartilaginous skeleton averages 50.5 per cent (range, 45.1 to 54.6 per cent). In the chronic series, the dry substance averages 49.2 per cent (range, 45.5 to 55.7 per cent). If case M 5 be excluded (which appears to be exceptional or erroneous), the average for the chronic series would be still lower, or 47.9 per cent. In any event, the dry content appears to be slightly lower and the water content correspondingly higher on the average in the chronic inanition series, which might be expected.

Data upon the normal composition of the skeleton of the rat for comparison are scarce. In one case which I have observed (at 10 weeks) the net body weight was 115 grams, ligamentous skeleton 9.91 per cent of body weight, fresh cartilaginous skeleton 5.57 per cent, and dry cartilaginous skeleton 2.97 per cent. In this case the dry substance formed 53.4 per cent of the cartilaginous skeleton. Lowrey ('13) in two albino rats (body weight 267.5 grams) finds the *ligamentous* skeleton forming about 9 per cent of the body weight, and containing 52.6 (52.1 to 53.1) per cent of dry substance. (This would probably be slightly higher than the dry content of the *cartilaginous* skeleton, however, on account of the higher percentage of water in the ligaments.) Further data are necessary before final conclusions can be reached, but it appears that in the rat (as in other animals) the percentage of water in the skeleton is increased during inanition.

It is a well known fact that in general the skeleton loses comparatively little during inanition, and thus increases greatly in relative (percentage) weight. In a series of six steers, the skeleton varied from 10.6 per cent of the body in a very fat animal to 19.3 per cent in a thin animal. (Data from the Missouri Agricultural Experiment Station.) An apparent slight loss in absolute weight of the (cartilaginous?) skeleton has been observed during inanition as follows: pigeons, by Chossat ('43), 3 per cent; cats, by Voit ('66), 14 per cent; little or none by Sedlmair ('99); dogs, little or none by Voit ('05 a) and Falck ('54); rabbits, little or none by Gusmitta ('84), Weiske ('95) and Pfeiffer ('87).

Since the calcified framework is but little affected in volume during inanition, it is evident that there can be but little, if any, loss in absolute weight of the skeleton. It is at least theoretically possible that there may even be a slight *increase* in its weight, since the fat in the bone-marrow is replaced by a mucoid substance (Jackson '04) presumably of higher specific gravity. Examination of bones, therefore, invariably shows a marked increase in water content during inanition. As Sedlmair ('99) states (p. 33): "Es giebt kein einziges sicheres Beispiel für einen geringeren Wassergehalt der Knochen hungernder Tiere; alle Forscher (Chossat und Lukjanow an Tauben, Gusmitta, C. Voit und Schöndorff an Hunden, Weiske an Kaninchen) fanden darin einen höheren Wassergehalt." This would naturally vary in different bones, and in different animals, according to the relative fat content. Small animals, like the rat, have in general relatively much less marrow fat than larger animals.

MUSCULATURE

The musculature (table 3; fig. 2) forms an average of 47.6 per cent of the body in the acute inanition series, which is slightly higher than the average for the normal adult rat (which is about 45 per cent, Jackson and Lowrey '12). The musculature in the chronic inanition series is somewhat lower, the average being 43.0 per cent of the body weight. This would indicate a somewhat greater loss relatively in chronic inanition.

In terms of absolute weight, the musculature would appear to have decreased from 114.75 to 79.25 grams (a loss of 30.9 per cent) in the acute inanition series; and from 96.30 to 57.0 grams (a loss of 40.8 per cent) in the chronic inanition series.

There is much variation in the weight of the musculature following inanition, judging from data available in the literature. The statements usually refer to acute inanition. According to Gaglio ('84), while the body weight of the frog loses 56 per cent the musculature loses 85 per cent of its weight. The musculature appears to suffer a slight relative loss (decrease in percentage weight) in dogs (Falck '54; Voit '05 b) and cats (Sedlmair '99), while it remains nearly unchanged in the rabbit (Pfeiffer '87; Voit, '05 b). On the other hand, data from a series of steers slaughtered in the Missouri Agricultural Experiment Station indicate a relative increase in the musculature from about 33 per cent of the body weight in a very fat animal, to 44 per cent in a lean animal, due probably to the earlier loss of the fat. Voit ('66) finds a slight relative increase in the musculature of the cat. Lasarew ('97) in an extensive series of guinea-pigs subjected to various degrees of inanition found that the loss of the musculature is somewhat less than that of the body fat in the earlier periods, but greater in the later periods. While the body weight lost 10 per cent, the musculature lost only 7.28 per cent of its weight; but the loss of the musculature was much greater at later periods. This would perhaps explain the relatively greater loss of the musculature during chronic inanition.

VISCERA AND REMAINDER

The average for the total visceral group in the normal adult rat, according to Jackson and Lowrey ('12; based upon only a few observations) is about 13.3 per cent of the body weight. In the acute nanition series (table 3; fig. 2) the average found is 11.1 per cent; while n the chronic series it is 13.4 per cent. This would seem to indicate that the loss in weight is relatively greater in acute than in chronic inanition. As a matter of fact, the great majority of the individual organs, as will be seen later, show a greater relative loss during chronic inanition. The liver and spleen are exceptions, however, and the large bulk of the former overbalances the other viscera when all are grouped together. On the whole, there is not much change in the relative weight of the visceral group; but there is, however, much variation among the individual organs, as will be seen later.

Aron ('11) states that the organs lose more than the musculature during inanition in dogs. Data by Voit ('05 b) show a decrease in the relative size of the viscera in the rabbit, but not much change in the dog.

The 'remainder' is the amount obtained by substracting from the net body weight the weight of the skin, skeleton, musculature and visceral group. It includes the loss by evaporation and escape of liquids, a few small unweighed organs, and the dissectable masses of fat. In the normal adult rat, the 'remainder' forms about 13 per cent of the body weight (Jackson and Lowrey '12). In the chronic inanition series, the average is 9.4 per cent. In the acute series the average is 7.3 per cent. The decrease in the relative weight of the 'remainder' is probably due chiefly to loss of fat.

BRAIN

The average for the brain in the acute inanition series (table 1) is 1.18 per cent of the body weight. By means of the tables and curves constructed by Donaldson ('09), the (theoretical) normal weight of the brain can be derived, corresponding to any given body weight. This gives an average of 0.78 per cent for the assumed normal weight of the brain at the beginning of the experiment (gross body weight, 244 grams). Since the average loss in body weight during the inanition period is one-third, it is evident that the brain has lost but little if any in absolute weight.

The theoretical average initial absolute weight of the brain is about 1.902 grams, forming 0.78 per cent of the (gross) body weight (Donaldson '09). According to Donaldson's ('08) table 1, the brain weight observed in six cases averaged 1.900 grams, the theoretical being 1.905 grams, at the body weight of 245 grams. If this absolute weight of the brain remained constant, while the body weight lost one-third (33.9 per cent), the final percentage weight of the brain would be increased about one-half, or to 1.17 per cent. The average absolute weight of the brain actually observed at the end of the period of acute inanition is 1.8046 grams, corresponding to an average of 1.18 per cent of the (net) body weight.[3] This weight is slightly less than would be expected if the brain remained constant in absolute weight, indicating a loss of about 5.1 per cent in the absolute weight of the brain during inanition.

The observations upon the brain in chronic inanition (table 3) indicate a similar condition. The average brain in this series weighs 1.743 grams and forms an average of 1.33 per cent of the (net) body weight. (This is the mean of the individual percentages; 1.743 grams would form 1.31 per cent of the average net body weight, 132.5 grams; or 1.28 per cent of the average corresponding gross body weight, 136 grams.) According to calculations from Donaldson's ('09) data, the average brain corresponding to the body weight at the beginning of the experiment (213.7 grams) should weigh 1.866 grams, forming about 0.87 per cent of the body weight (the percentage being higher than in the acute inanition series on account of the smaller average body weight).[4] This is slightly higher than the result to be expected on the assumption that the absolute brain weight has remained

[3] This is the mean of the individual percentages, as shown in table 3. The average brain weight, 1.8046 grams, would correspond to 1.13 per cent of the corresponding average gross body weight, 160 grams, at the end of the experiment.

[4] According to Donaldson's ('08) table 1, corresponding to a body weight of 215 grams, the average of 8 brains, both sexes, was 1.873 grams, the corresponding theoretical weight by formula being 1.871 grams.

constant, while the body weight has decreased about one-third (36.1 per cent). In other words, the results indicate a loss of about 6.6 per cent in the absolute weight of the brain during chronic inanition, which is slightly greater than the apparent loss (5.1 per cent) during acute inanition. A larger series of observations would be necessary, however, to draw any final conclusions with precision.

The data agree fairly well with the results of Hatai ('04) who found an estimated loss of about 5 per cent in the weight of the brain of young rats during chronic inanition. Donaldson ('11), however, found in still younger rats an actual *increase* of 3.6 per cent in the brain weight during chronic inanition (body weight held constant from age of 30 days to 51 days); while my own observations (Jackson '15) indicate little or no change in the brain weight under these conditions.

It has long been known that of all the organs of the body, the central nervous system apparently suffers least in weight (if at all) during inanition. Thus Chossat ('43) found no loss[6] in the weight of the brain in starved pigeons, with loss in body weight of about 40 per cent. Falck ('54) in dogs, and Lasarew ('97) in guinea-pigs, found little or no loss in the weight of the brain and cord during inanition. Bowin ('80) and Pfeiffer ('87) found an absolute decrease (but relative increase) in the brain weight of the rabbit. Voit ('66) in cats found a decrease of about 3 per cent in the weight of brain and cord; while the data of Sedlmair ('99) would even indicate an actual *increase* in their weight during starvation. Data from a series of steers slaughtered in the Missouri Agricultural Experiment Station show that the absolute weight of the brain and cord in thin animals is but very slightly less than in very fat animals with nearly double the body weight. The relative (percentage) weight is of course correspondingly higher in the thin animals.

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[6] Since Chossat's results are often misquoted, it may be noted that he found the average weight of the brain almost identical in the 8 starved pigeons and in 8 controls of nearly the same (initial) body weight; (brain weight average 2.27 grams for starved; 2.25 grams for corresponding controls). On account of the difficulty in determining the exact plane of separation between the brain and spinal cord, however, Chossat preferred to combine their weights, thus giving a slight decrease (about 1.9 per cent) in the absolute weight of the entire central nervous system during inanition.

In young animals during the period of active growth, the results of inanition (especially chronic inanition) are in general somewhat different from those in adults, since the tendency of the organs to maintenance is in the young animal complicated by the growth impulse (Jackson '15). Bechterew ('95) found a slight apparent loss in the absolute weight of the brain and spinal cord of the newborn cat and dog during acute inanition. Hatai ('04) found in a series of young albino rats subjected to chronic inanition for 21 days an estimated average loss of about 5 per cent in the absolute weight of the brain. Later (Hatai '08) in another group of young rats in which growth had been retarded by underfeeding, he found that in these stunted animals the brain and cord had a weight approximately normal for the corresponding body weight. Donaldson ('11), in still younger rats held at nearly constant body weight by underfeeding from age of 30 days to 51 days, finds an apparent *increase* of 3.6 per cent in the weight of the brain. My own observations (Jackson '15), however, indicate little or no change in the weight of the brain under these conditions.

On the whole it appears that in adults inanition, both acute and chronic, produces a slight loss in the absolute weight of the brain. In young, rapidly growing animals, however, it is doubtful whether any such loss occurs, especially in chronic inanition, where there is even a possible increase in brain weight.

SPINAL CORD

The spinal cord in the acute inanition series (table 3) has an average weight of 0.631 gram and forms an average of 0.41 per cent of the body weight. Calculations from Donaldson's ('09) data indicate that at the average initial gross body weight (244 grams) the spinal cord should weight 0.625 gram, forming 0.26 per cent of the body weight. The data would therefore seem to indicate not only no loss, but even a very slight *gain* of the spinal cord in absolute weight during the period of acute inanition. In an earlier paper, Donaldson ('08) in table 4 gives the average weight of the spinal cord (at body weight of 245 grams) as 0.630 gram in 2 males, and 0.640 gram in 4 females. On the whole, therefore, we may conclude that the spinal cord during acute inanition undergoes little or no change in weight. Individual variations make comparisons with controls more or less uncertain, especially in a relatively small series of observations.

A somewhat different result is found in the chronic inanition series. Donaldson's ('09) data would indicate that the spinal cord in body weights corresponding to the average initial weight (214 grams) of this series would form an average weight of 0.593 gram or 0.28 per cent of the body weight. In an earlier paper, Donaldson ('08) in table 4 gives the weight of the spinal cord (at body weight of 215 grams) as averaging 0.590 gram in 5 males, and 0.630 gram in 3 females (corresponding theoretical weight by formula being 0.593 gram, sexes combined). The actual weight of the cord found at the end of the inanition period averages 0.569 gram, or about 0.43 per cent of the body weight. This would indicate a loss of about 4 per cent in the absolute weight of the spinal cord during chronic inanition. Thus in the spinal cord, as in the brain, there appears to be in the adult rat a tendency to greater loss in chronic than in acute inanition. In the young rat, however, this tendency is more than counterbalanced by the growth impulse, so that the spinal cord may gain in weight while the body weight is held constant (Donaldson '11; Jackson '15).

In any event, however, it is evident that the adult spinal cord, like the brain, shows but very slight if any loss in absolute weight during inanition, thus increasing markedly in relative (percentage) weight. This is in general agreement with the observations of Chossat ('43), Falck ('54), Lasarew ('97), Sedlmair ('99), Voit ('66), and Bechterew ('95) previously mentioned in the discussion of the brain.

EYEBALLS

The eyeballs in the acute inanition series (table 3) form an average of about 0.19 per cent of the body weight. In normal rats corresponding to their initial body weight, the average should be about 0.12 per cent (Jackson '13). Since the body weight has decreased about one-third (average 33.9 per cent) it is evident that the eyeballs must have remained nearly stationary in absolute weight, thus increasing their relative (percentage) weight by about one-half. In terms of absolute weight, there would appear to be a reduction from 0.298 gram (the theoretical weight corresponding to the initial body weight of 244 grams, according to Hatai '13) to 0.285 gram, the final average weight of the eyeballs (table 3). This would indicate a loss of 4.4 per cent.

A similar condition is found in the chronic inanition series. The eyeballs here form an average of about 0.20 per cent of the body weight, while the normal for the initial body weight averages about 0.13 per cent. (The higher figures in the chronic inanition series are due to the smaller initial body weight in this series.) Thus it is evident that, with a loss of about one-third (average 36.1 per cent) in the body weight during inanition, the absolute weight of the eyeballs has changed but little. In terms of absolute weight there would appear to be a reduction from about 0.280 gram (the theoretical weight corresponding to the average initial body weight of 214 grams, according to Hatai '13) to 0.2638 gram, the final average weight of the eyeballs (table 3). This would correspond to a loss of 5.8 per cent in the weight of the eyeballs during chronic inanition.

That the eyeballs lose little or nothing in absolute weight during inanition, increasing proportionately in relative (percentage) weight, is also indicated by the data of Falck ('54) for the dog, and Sedlmair ('99) for the cat. Bitsch ('95) finds the (absolute) weight of the eyeballs in the dog usually *increased* during inanition, which he suggested may be due to oedema.

THYROID GLAND

The thyroid gland in the acute inanition series (table 3) forms an average of 0.023 per cent of the body weight. The normal for the initial body weights would average about 0.015 per cent (Jackson '13). Since the body weight has been reduced one-third during inanition, this would indicate that there has been no loss in the absolute weight of the thyroid gland. In terms of absolute weight, the thyroid gland after acute inanition averages 0.0380 gram, which is almost identical with the theoretical weight corresponding to the average initial body weight of 244 grams (Hatai '13).

The results in chronic inanition are somewhat different. Here the thyroid gland forms an average of about 0.020 per cent of the body weight, the normal for the corresponding initial body weights being about 0.016 per cent. In this case the thyroid gland has apparently lost weight during chronic inanition, though relatively less than the body as a whole. According to Hatai ('13) the weight of the thyroid gland corresponding to the average initial body weight of 214 grams would be about 0.034 gram. The final absolute weight averages 0.0266 gram, indicating a loss of about 21.8 per cent in chronic inanition.

On account of the variability of the thyroid gland, however, and the difficulty in dissecting it out accurately, no final conclusions can be drawn from the limited number of observations at hand. As in the case of the brain, spinal cord and eyeballs, however, there appears to be a greater tendency to loss in weight of the thyroid gland during chronic inanition.

Excepting the observations by Falck ('54), indicating no change in the relative (percentage) weight of the thyroid gland during inanition in the dog, no data on this subject have been found in the literature. Traina ('04) cites data, however, upon the histological changes.

THYMUS

It is already known that the thymus in the albino rat normally undergoes a diminution in weight (due to age involution) after the age of 10 weeks (Jackson '13) or more precisely 85 days (Hatai '14). At the age of one year, it forms normally about 0.020 per cent of the body (Jackson). The thymus in the present acute inanition series forms an average of about 0.020 per cent of the body weight, while in the chronic inanition series the average is 0.021 per cent (table 3). The age of the rats used in the inanition experiments is unknown, but from the body weight it is probable that few of them were less than a year old. Therefore while hunger is known to produce a marked involution of the thymus (Hammar), it is probable that in the present case the involution had already been produced by age, and was not caused by inanition. According to Jonson ('09), in young rabbits the weight curves of fat and thymus are similar in *chronic* inanition, but the fat decreases somewhat more rapidly in *acute* inanition.

HEART

The heart in the acute inanition series forms an average of about 0.43 per cent of the body weight, and about 0.42 per cent in the chronic inanition series (table 3). The normal for corresponding initial body weights would average about 0.43 per cent (Jackson '13) or about 0.38 per cent, according to Hatai's ('13) data. Thus it is evident that the heart during inanition has lost weight nearly in the same proportion as the whole body. Using Hatai's ('13) curves to determine the normal at corresponding initial body weights, the absolute weight of the heart apparently decreases from about 0.925 to 0.6687 gram (loss of 27.7 per cent) in the acute inanition series; and from about 0.830 to 0.5577 gram (loss of 32.8 per cent) in the chronic inanition series. As in the case of the viscera previously considered, the loss is apparently slightly greater relatively in chronic inanition.

The statements in the literature generally indicate that the heart during inanition loses somewhat less in weight than the body as a whole, and increases accordingly in relative (percentage) weight. This would appear to be the case in man (Aschoff '11; Lustig '02), cat (Voit '66; Sedlmair '99), newborn kitten (v. Bechterew '95) dog (Falck '54), guinea-pig (Lasarew '97), rabbit (Bowin '80), and in thin compared with fat steers (data from Missouri Agricultural Experiment Station). The data of Chossat ('43) for pigeons, however, would indicate a reduction in heart weight relatively slightly greater than in the body as a whole.

LUNGS

The lungs of the rat are quite variable in weight, owing to the frequency of infection, which may affect their weight even when the lesions are slight. The average for the lungs in the acute inanition series is 0.61 per cent of the body weight, and 0.55 per cent in the chronic inanition series (table 3). Since the normal lungs corresponding to the average initial body weight form an average of about 0.60 per cent (Jackson '13), it appears (although the evidence is insufficient for final conclusions) that during inanition, both acute and chronic, in adult albino rats the lungs lose in weight in about the same proportion as the whole body, their relative (percentage) weight remaining nearly the same. Taking the normal weight of the lungs at corresponding body weights from Hatai's ('13) curve, there is apparently a decrease from about 1.40 to 0.968 gram (loss of 30.9 per cent) in the acute inanition series; and from about 1.24 to 0.743 gram (loss of 40 per cent) in the chronic series. Again, as in the case of the viscera previously considered, the loss appears relatively greater during chronic inanition.

Comparatively few data are found in the literature concerning the weight of the lungs in inanition. In pigeons, Chossat ('43) found a loss of 22.4 per cent in the lungs, compared with 40 per cent in the entire body weight. Thus their relative (percentage) weight is considerably increased, which was also observed by Voit ('66) and Sedlmair ('99) in the cat, and von Bechterew ('95) in newborn kitten. In the dog, however, Falck's ('54) data indicate a loss relatively slightly greater than that of the body, with a corresponding decrease in percentage weight.

LIVER

The liver in the acute inanition series (table 3) forms an average of about 2.88 per cent of the body weight. Since the normal for the corresponding initial body weights is about 4.5 per cent in the acute inanition series, and 4.3 per cent in the chronic (Jackson '13) it is evident that the liver has apparently lost in weight relatively more than the body as a whole. In the chronic inanition series, however, the weight of the liver is more variable, and the average (3.98 per cent) is but little below the normal. As the liver is normally subject to great individual variations in weight (Jackson '13), however, caution should be observed in drawing final conclusions.

In terms of absolute weight, using my (Jackson '13) data for comparison, it appears that the liver decreases from about 10.98 to 4.587 grams (loss of 58 per cent) in the acute inanition series, and from 9.20 to 5.219 grams (loss of 43 per cent) in the chronic inanition series. It may also be remembered that in the series investigated by Hatai ('13), the average weight of the normal liver was found distinctly higher than in my series; and the loss in weight, estimated upon this basis, would be considerably greater.

With the exception of the adipose tissue, the thymus (in young animals) and occasionally the spleen, all investigators agree that the loss of weight in the liver is relatively greater than that in any other organ. Thus the liver decreases in relative (percentage) weight, as has been observed in man (Aschoff '11), white mouse (Cesa-Bianchi '09), pigeon (Chossat '43), rabbit (Pfeiffer '87), cat (Voit '66; Sedlmair '99), dog (Falck '54), newborn cat and dog (v. Bechterew '95), guinea-pig (Lasarew '97) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). While the loss is practically always relatively greater than in the body as a whole, the amount of loss is quite variable.

Since the loss in weight of the liver (unlike all viscera previously considered) is relatively greater in acute inanition it seems probable that the greatest loss occurs during the earliest stages of inanition, when the liver yields its store of easily available food material (glycogen, fat, etc.). Thus Lasarew ('97) found that during the early period of inanition in the guinea-pig, while the body weight lost 10 per cent, the liver lost 18 per cent of its weight, or relatively nearly twice as much. Toward the end of the inanition period, on the contrary, the liver apparently lost relatively only half as much as the whole body.

SPLEEN

In the acute inanition series (table 3), the spleen forms an average of 0.21 per cent of the body. This is below the normal for the average corresponding initial body weight, in which case the average is 0.27 per cent. In the chronic inanition series, however, the average (0.30 per cent) is slightly higher than the normal (Jackson '13). The individuals in both acute and chronic inanition, however, are exceedingly variable, as seen in the table. This is also true of the normal spleen, which is one of the most variable organs in the body (Jackson '13). The number of observations is therefore insufficient for final conclusions concerning the effect of inanition upon the weight of the spleen in the albino rat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the spleen from about 0.645 to 0.3177 gram (loss of 51 per cent) in the acute inanition series; and from 0.570 to 0.4056 gram (loss of 29 per cent) in the chronic series.

That the spleen loses heavily during inanition, losing in relative as well as absolute weight, has been found in man (Aschoff '11; Stschastný '98), pigeon (Chossat '43), rabbit (Bowin '80), and cat (Voit '66; Sedlmair '99); while a decrease less marked than in the whole body (relative increase) appears in the dog (Falck '54) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). Data from von Bechterew ('95) indicate a relative increase in the spleen of newborn kittens during inanition, but a decrease in puppies. These apparently conflicting statements are perhaps to be explained largely by the great variability of the spleen, making comparison with controls uncertain. In addition, however, there is the possibility that the loss in the spleen may vary according to the character and stage of inanition. Thus Lasarew ('97) in the guinea-pig found the greatest loss in weight of the spleen to occur in the middle period of inanition (second period of 10 per cent loss in body weight), during which the spleen lost 31 per cent in weight.

STOMACH AND INTESTINES

The stomach and intestines (including mesentery and pancreas) together with their content (table 3) form an average of about 5.2 per cent of the body weight in the acute inanition series, and 6.3 per cent in the chronic inanition series. Since the average normal for the initial body weights is about 9.0 per cent (Jackson '13), it is evident, as might be expected, that the stomach and intestines with contents have lost weight in relatively much greater proportion than has the body as a whole. The loss affects not only the contents, but the empty tract, which has apparently decreased from about 6.0 per cent (normal at the initial body weight) of the body weight to 3.25 per cent (acute inanition series) or 3.5 per cent (chronic series). There is apparently not much difference in this respect between the chronic and acute series. A relatively small part of the loss is in the mesenteric fat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the empty alimentary canal from about 11.65 to 5.01 grams in the acute inanition series, and from 10.70 to 4.54 grams in the chronic series. This would correspond to a loss of about 57 per cent in each series.

Data in the literature are very scarce concerning changes in weight of the alimentary canal during inanition. According to Falck ('54), the loss appears to be nearly proportional to that of the entire body (relative weight increasing from 8.4 to 8.7 per cent). This appears to be true also for the cat according to Sedlmair ('99), although a much smaller loss was found by Voit ('66). Unpublished data on a series of steers (Missouri Agricultural Experiment Station) indicate that the relative weight of the (empty) intestines (without fat) is variable, but tends to be greater in thin than in fat animals.

SUPRARENAL GLANDS

The suprarenal glands must be considered separately in the sexes on account of a difference in relative weight (Jackson '13). In normal rats corresponding to the initial body weights of the present series, the average for the suprarenal glands of the male is about 0.17 per cent of the body weight, and for the female about 0.26 per cent. The four females of the acute inanition series (none present in the chronic series) show an average of 0.30 per cent of the body weight (table 3), but the number is too small for definite conclusions. The males show an increase (from 0.17 per cent) to an average of 0.25 per cent in both acute and chronic inanition series (table 3). It would therefore appear that the loss in absolute weight has been less than in the body as a whole in the female with little or no loss in the male.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, it appears that in the male rat the suprarenals have *increased* from about 0.0390 to 0.0396 grams (gain of 1.5 per cent) in the acute inanition series; and *decreased* from about 0.0360 to 0.0328 grams (loss of 8.9 per cent) in the chronic series. The apparent increase during acute inanition is probably not significant. A larger number of observations is necessary to determine the matter.

No data concerning the weight of the suprarenal glands during inanition have been found in the literature, although Traina ('04) cites several investigations on the histological changes.

KIDNEYS

The kidneys (table 3) in the acute inanition series form an average of 0.97 per cent of the body weight and in the chronic series 1.00 per cent. According to Jackson ('13) the normal for the initial body weights would average about 0.95 per cent (although Hatai's data would place it at about 0.84 per cent). It therefore appears that the kidneys have lost weight to nearly the same extent relatively as the body in general, thus gaining but slightly in relative (percentage) weight.

If Hatai's ('13) curve be taken as the normal, in absolute weight the kidneys would apparently decrease from about 2.04 to 1.520 grams (a loss of 25.5 per cent) during acute inanition; and from 1.80 to 1.317 grams (a loss of 26.8 per cent) in the chronic inanition series. If my data indicating a higher normal were taken, the loss would be correspondingly greater.

Considering the importance of the kidneys, there is in the literature a surprising lack of data concerning their weight during inanition. That they lose in weight relatively somewhat less than the body as a whole (thus gaining in percentage weight) is indicated in the pigeon (Chossat '43), cat (Voit '66) and dog (Falck '54). There appears also a slight relative increase in the kidney weight of thin steers compared with fat (data from Missouri Agricultural Experiment Station). On the other hand, Sedlmair's ('99) data for starved cats indicate no change in relative (percentage) weight in one case and a slight decrease in another.

GONADS

a. Female. The two observations recorded for the weight of the ovaries, 0.051 per cent and 0.033 per cent of the body weight are both above the normal average (0.025 per cent), but are of course entirely too few to be significant.

b. Male. At the age of one year (body weight, 210 grams) the normal testes and epididymi form an average of about 1.20 per cent of the body weight, of which approximately one-fourth (0.30 per cent) belongs to the epididymi, and three-fourths (0.90 per cent) to the testis (Jackson '13). Hatai's ('13) data would put the testis a little higher (about 1.05 per cent). In the acute inanition series (table 3) the average relative weight for the testes is 1.12 per cent, and for the epididymi is 0.39 per cent of the body weight. In the chronic series, the average for the testes is 1.02 per cent, and for the epididymi 0.33 per cent. It would therefore appear that during inanition the loss in weight of both testes and epididymi is relatively not very different from that in the body as a whole, and is (like that of the majority of the viscera) more marked in chronic than in acute inanition. On account of the variability of these organs, however, more data are needed before final conclusions can be justified.

In terms of absolute weight, taking Hatai's ('13) data for the normal at the initial body weight, the testis would appear to decrease from 2.50 to 1.756 grams (a loss of 29.8 per cent) in the acute inanition series; and from 2.27 to 1.355 grams (a loss of 40.3 per cent) in the chronic inanition series.

Although numerous investigations have been made upon the histological changes in the gonads during inanition (Traina '04), the only observations found concerning their weight are that by Falck ('54) showing the relative (percentage) weight of the testis in the dog to remain apparently unchanged, and that by (Voit '66) showing a relative decrease in the testis of the cat.

HYPOPHYSIS

In the case of the hypophysis, as with the suprarenal glands, there is normally a sexual difference to be considered (Hatai '13). When the data in table 3 are compared with Hatai's chart 10, it will be found that the absolute weights of the hypophysis, in both acute and chronic inanition, correspond fairly well with those of the normal gland at the *final* body weight. That is, the weight of the hypophysis during inanition has apparently decreased in nearly the same proportion as the whole body, so the relative weight is but little changed. As calculated from Hatai's data, the (male) hypophysis would form about 0.0036 per cent of the body at the average initial body weight, as compared with 0.0043 per cent found in the acute inanition series, and 0.0045 per cent in the chronic series.

In terms of absolute weight, the (male) hypophysis has apparently decreased from about 0.0093 to 0.0069 gram, the average of the acute inanition series (loss of 26.1 per cent); and from 0.0079 to 0.0059 gram (loss of 25.3 per cent) in the chronic inanition series. A larger number of observations would of course be necessary to determine the result with precision.

No data have been found in the literature concerning the weight of the hypophysis during inanition.

DISCUSSION

The changes in the average relative weights of the various organs and systems as a result of inanition in the adult albino rat are summarized in table 2.

While no great emphasis can be laid upon the exactness of the figures shown in table 2, it is evident that with respect to relative loss in weight during inanition, the organs may be divided into three groups. In the first group, which includes the suprarenals, thyroid, skeleton, eyeballs, spinal cord and brain, and thymus, there is but little (if any) loss in absolute weight during inanition, and a corresponding increase in relative (percentage) weight. In general, there is a relatively greater loss during chronic

TABLE 2

ORGAN OR SYSTEM	NORMAL PERCENTAGE OF BODY AT INITIAL BODY WEIGHT	PERCENTAGE OF BODY AFTER INANITION		PERCENTAGE LOSS OF ORGAN DURING INANITION	
		Acute	Chronic	Acute	Chronic
Suprarenals (male)	0.0170	0.0220	0.0260	+1.5(?)	−8.9
Thymus	0.0200	0.0200	0.0210	0(?)	0(?)
Thyroid gland	0.0150(0.016)	0.0230	0.0200	0(?)	−21.8
Spinal cord	0.2500(0.290)	0.4000	0.4300	0(?)	−4.0
Ligamentous skeleton	10.0000	15.0000	16.4000	−0.4	+1.8(?)
Eyeballs	0.1200(0.130)	0.1900	0.2000	−4.4	−5.8
Brain	0.7800(0.870)	1.1700	1.3300	−5.1	−6.6
Kidneys	0.9500	0.9600	1.0000	−25.5	−26.8
Hypophysis	0.0036	0.0043	0.0045	−26.1	−25.3
Heart	0.4300	0.4400	0.4200	−27.7	−32.8
Testes	0.9000	1.0600	1.0100	−29.8	−40.3
Lungs	0.6000	0.6100	0.5500	−30.9	−40.0
Musculature	45.0000	47.5000	43.0000	−30.9	−40.8
Integument	18.0000	19.1000	17.8000	−31.2	−38.5
Whole body	*100.0000*	*100.0000*	*100.0000*	*−33.9*	*−36.1*
Spleen	0.2700	0.2100	0.3100	−51.0	−29.0
Stomach—intestines	6.0000	3.4000	3.5000	−57.0	−57.0
Liver	4.5000	3.1000	4.0000	−58.0	−43.0

inanition, which is especially marked in the case of the thyroid gland. The thymus, having already undergone age involution, is affected but slightly, if at all.

In the second group, which includes the kidneys, hypophysis, heart, testes, lungs, musculature and integument, the loss in absolute weight during inanition is more nearly in proportion to that of the whole body, so their relative (percentage) weight is usually not greatly changed. In all except the hypophysis, however, the loss is relatively greater during chronic inanition. Especially the lungs, testes, integument and musculature appear to lose markedly during chronic inanition.

In the third group, including the spleen, liver and alimentary canal, the loss in absolute weight is relatively much greater than in the body as a whole, so they decrease in relative as well as in absolute weight. The liver and spleen are exceptional, however, in that their loss is apparently relatively greater in acute than in chronic inanition. In fact, in chronic inanition the spleen apparently belongs with the second group.

The variability of the organs as to loss of weight during inanition has been explained in two ways: Manassein ('69) noted that those organs which are most active in the organism lose least during inanition. A more rational explanation is that of Paschutin ('81), according to whom the various organs lose in proportion to their storage content of available food supply. The various proteids, fats and carbohydrates are dissolved and carried away by the circulation at different times and with different degrees of rapidity. Thus the variability in the loss of weight in different organs and in different types of inanition would be ultimately explained primarily upon a chemical basis.

SUMMARY

The principal results of the present paper may be briefly summarized as follows:

1. During both acute and chronic inanition there is apparently a slight increase in the ratio of tail length to body length. This is probably due to a decrease in the trunk length during inanition.

2. The head and fore limbs during inanition lose relatively less than the body as a whole, and therefore increase in relative (percentage) weight. The hind limbs nearly maintain their original relative weight (slight increase during acute inanition), while the trunk decreases in relative weight.

3. Of the systems—integument, skeleton, musculature, viscera and 'remainder'—the integument and musculature lose relatively in nearly the same proportion as the whole body, slightly less during acute inanition and slightly more during chronic inanition. The skeleton nearly maintains its original absolute weight, and therefore increases markedly in relative (percentage) weight. There is a marked decrease in the 'remainder,' probably due chiefly to loss of fat. The visceral group as a whole undergoes little change in relative weight, showing a slight decrease during acute inanition. This decrease is due to the large size of the liver, which undergoes a greater loss in acute than in chronic inanition. The majority of the viscera, on the other hand, show a greater loss during chronic inanition.

4. As to relative loss of weight during inanition, the individual viscera may be divided into three groups: (1) the suprarenal glands, thyroid glands, eyeballs, spinal cord and brain lose but very little (if any) in absolute weight, and therefore increase correspondingly in relative (percentage) weight. The thymus has already undergone age involution, and is therefore unaffected. (2) The kidneys, heart, lungs, hypophysis and testes lose more nearly in proportion to the entire body (in general, somewhat more during chronic inanition), and therefore do not change greatly in relative (percentage) weight. (3) The spleen (in acute inanition), liver and alimentary canal (both empty and with contents) lose relatively much more heavily than the whole body, and therefore decrease in relative (percentage) as well as in absolute weight.

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TABLE 3

Acute and chronic inanition

RAT NO. AND SEX	LENGTH OF BODY	LENGTH OF TAIL	RATIO OF TAIL TO BODY	INANITION PERIOD	ORIGINAL GROSS BODY WEIGHT	FINAL GROSS (AND NET) BODY WEIGHT	LOSS OF BODY WEIGHT	HEAD		FORE-LIMBS	
								Weight	Percent of body	Weight	Percent of body
	cm.	*cm.*		*days*	*grams*	*grams*	*per cent*	*grams*		*grams*	
S 18 f.	17.5	17.5	1.00	Acute, 8.0	196.0	121.0(117.2)	38.0	16.2	13.9		
M 8 m.	17.1	17.3	1.01	10.0	221.0	132.0(128.9)	40.0	14.1	10.9	9.2	7.1
O 6 f.	18.0	15.8	0.88	12.0	193.0	132.0(129.5)	32.0	17.4	13.4		
M 7 m.	16.6	16.0	0.96	9.0	216.0	134.0(131.2)	38.0	14.2	10.8	8.5	6.5
M 9 m.	15.3	16.5	1.08	6.0	190.0	137.0(134.0)	28.0	14.8	11.1	8.7	6.5
O 5 f.	20.0	18.0	0.90	8.0	182.0	136.0(135.0)	25.0	16.4	12.1		
M 10 m.	18.1	17.2	0.95	9.0	221.0	142.0(139.4)	36.0	14.8	10.7	10.3	7.4
M 1 m.	20.5	19.5	0.95	9.0	248.0	168.0(165.2)	32.0	17.0	10.3	13.8	8.4
M 2 m.				11.0	254.0	170.0(167.2)	33.0	18.9	11.3	12.7	7.6
S 26 m.	20.5	18.0	0.88	8.0	284.0	174.0(171.5)	39.0	18.7	10.9		
S 16 f.	19.5	17.5	0.90	9.0	267.0	190.0(186.0)	29.0	20.3	10.9		
S 25 m.	20.5	19.0	0.93	9.0	328.0	202.0(198.3)	35.0	22.1	11.1		
S 27 m.	21.5	18.0	0.84	8.0	320.0	223.0(219.0)	30.0	23.6	11.0		
M 13 m.	21.3	19.6	0.92	6.0	333.0	240.0(237.0)	28.0	23.7	10.0	16.8	7.1
S 28 m.	23.0	19.5	0.85	10.0	367.0	243.0(240.0)	34.0	24.6	10.3		
Average (acute)	19.2	17.8	0.93	Acute, 9.0	255.0 (244 for viscera)	170.0(166.0) 160.0(157.4) for viscera	33.1 (33.9 for viscera)	18.47	11.2 (10.7 for limbs and trunk	11.4	7.2
M 3 m.	17.5	16.5	0.94	Chronic, 36.0	188.0	125.0(122.1)	33.5	13.9	11.4	9.8	8.0
M 12 m.	17.3	17.1	0.99	33.0	200.0	128.0(124.6)	36.0	14.2	11.4	7.8	6.2
M 5 m.	19.0	18.5	0.97	36.0	205.0	129.0(126.6)	37.0	15.2	12.0	10.4	8.2
M 4 m.	17.5	17.0	0.97	41.0	205.0	134.0(129.2)	35.1	13.5	10.5	7.4	5.7
M 6 m.	17.5	16.7	0.95	35.0	220.0	138.0(134.1)	37.2	15.3	11.4	9.5	7.1
M 11 m.	19.0	18.6	0.98	33.0	264 0	163.0(158.5)	37.5	18.3	11.5	9.8	6.2
Average (chronic)	18 0	17 4	0 97	Chronic, 35 7	213 7	136 2(132 5)	36 1	15 1	11 4	9 1	6 9

TABLE 3—*Continued*

RAT NO. AND SEX	HIND-LIMBS		TRUNK		INTEGUMENT		LIGAMENTOUS SKELETON		CARTILAGINOUS SKELETON		DRY SKELETON	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.					23.06	19.7	20.30	17.4				
M 8 m.	22.5	17.4	83.1	64.5	25.50	19.8	21.60	16.7	16.15	12.5	7.321	5.68
O 6 f.					24.20	18.6	19.50	15.1				
M 7 m.	23.9	18.2	84.6	64.5	24.80	18.9	19.70	15.4	15.19	11.6	7.224	5.51
M 9 m.	23.8	17.8	86.7	64.7	25.20	18.8	19.50	16.4	14.81	11.1	7.094	5.30
O 5 f.					22.00	16.3	15.20	11.3				
M 10 m.	24.3	17.5	90.0	64.7	27.30	19.6	21.70	15.6	15.05	10.8	7.325	5.27
M 1 m.	28.6	17.3	105.8	64.1	32.20	19.5	26.00	15.8	19.30	11.7	10.516	6.37
M 2 m.	27.6	16.5	108.0	64.6	35.00	21.0	21.00	12.6	14.00	8.4	7.741	4.64
S 26 m.					33.36	19.5	29.34	17.1				
S 16 f.					32.48	17.5	28.13	15.1				
S 25 m.					39.78	20.2	25.48	12.9				
S 27 m.					40.63	18.5	33.03	15.1				
M 13 m.	42.6	18.0	153.9	64.9	46.70	19.8	37.60	15.9	24.37	10.3	13.324	5.62
S 28 m.					41.43	17.3	40.01	16.7				
Average (acute)	27.6	17.5	101.7	64.6 (64.1)	31.60	18.9 (19.1 for viscera)	25.40	15.3 (15.0 for viscera)	16.98	10.9	8.748	5.48
M 3 m.	18.5	15.2	79.9	65.5	19.50	16.0	18.50	15.2	14.00	11.5	6.511	5.34
M 12 m.	20.7	16.6	81.9	65.5	21.40	17.1	21.40	17.1	14.60	11.7	7.156	5.72
M 5 m.	19.0	15.0	82.0	64.6	24.50	19.3	22.50	17.8	16.65	13.2	9.310	7.33
M 4 m.	15.9	12.3	92.4	71.4	18.90	14.7	18.20	14.1	16.10	12.5	7.318	5.67
M 6 m.	21.1	15.7	88.2	65.8	25.50	19.1	21.90	16.3	16.20	12.1	7.690	5.74
M 11 m.	26.5	16.7	103.9	65.3	32.70	20.6	28.30	17.8	21.52	13.5	11.015	6.38
Average (chronic)	20.3	15.3	88.05	66.4	23.70	17.8	21.80	16.4	16.5	12.4	8.167	6.03

TABLE 3—*Continued.*

RAT NO. AND SEX	MUSCULATURE		TOTAL VISCERA		REMAINDER		BRAIN		SPINAL CORD		EYEBALLS	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	55.64	47.6	12.82	11.0	5.37	4.3	1.833	1.57	0.5800	0.50	0.2660	0.23
M 8 m.	59.60	46.2	14.03	10.9	8.20	6.4	1.704	1.32	0.5652	0.44	0.2594	0.20
O 6 f.	58.70	45.3	14.12	10.9	13.08	10.1	1.802	1.39	0.6340	0.49	0.3180	0.25
M 7 m.	66.90	51.0	13.55	10.3	6.20	4.4	1.738	1.33	0.5710	0.44	0.2124	0.16
M 9 m.	67.80	50.6	(viscera not weighed)									
O 5 f.	65.80	48.7	18.60	13.8	13.40	9.9	1.650	1.22	0.5520	0.41	0.3120	0.23
M 10 m.	65.3	47.0	16.30	11.8	8.80	6.2	1.768	1.27	0.5794	0.42	0.2416	0.17
M 1 m.	75.00	45.5	16.80	10.2	13.70	8.7	1.734	1.05	0.6010	0.36	0.2838	0.17
M 2 m.	78.10	46.8	17.90	10.7	14.50	8.7	1.842	1.10	0.5542	0.33	0.2942	0.18
S 26 m.	79.49	46.4	16.50	9.6	12.70	7.4	1.773	1.04	0.7200	0.42	0.2470	0.15
S 16 f.	91.80	49.4	23.25	12.5	10.23	5.5	1.811	0.97	0.6990	0.38	0.3206	0.17
S 25 m.	99.02	50.0	18.22	9.2	15.25	7.7	2.005	1.01	0.7780	0.39	0.3222	0.16
S 27 m.	100.10	45.7	25.60	11.7	21.90	10.0	1.995	0.91	0.7380	0.34	0.3440	0.16
M 13 m.	122.00	51.4	(viscera not weighed)									
S 28 m.	103.50	43.7	(viscera not weighed									
Average (acute)	79.25	47.6 (47.5)	17.30	11.1	10.30	7.3	1.8046	1.18	0.6310	0.41	0.285	0.19
M 3 m.	54.20	44.4	19.34	16.0	10.50	8.4	1.794	1.47	0.5840	0.48	0.2580	0.21
M 12 m.	56.50	45.2	15.78	12.6	9.50	7.7	1.707	1.37	0.5394	0.43	0.2680	0.21
M 5 m.	54.30	42.8	15.27	12.1	10.00	8.0	1.704	1.34	0.5990	0.47	0.2990	0.24
M 4 m.	51.10	39.6	18.60	14.5	22.40	16.9	1.852	1.44	0.6060	0.47	0.2368	0.18
M 6 m.	58.60	43.7	18.61	13.9	9.50	7.1	1.578	1.18	0.4950	0.37	0.2548	0.19
M 11 m.	67.40	42.4	17.49	11.0	12.60	8.2	1.821	1.15	0.5908	0.37	0.2662	0.17
Average (chronic)	57.00	43.0	17.51	13.4	12.40	9.4	1.743	1.33	0.5690	0.43	0.2638	0.20

TABLE 3—*Continued.*

RAT NO. AND SEX	THYROID GLAND		THYMUS		HEART		LUNGS		LIVER		SPLEEN	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	0.0276	0.024	0.0072	0.006	0.5690	0.49	0.7870	0.67	2.921	2.50	0.1150	0.10
M 8 m.	0.0320	0.025	0.0326	0.025	0.5170	0.40	0.7440	0.58	2.714	2.10	0.2420	0.19
O 6 f.					0.7210	0.56			4.457	3.44	0.3590	0.28
M 7 m.	0.0290	0.022	0.0331	0.025	0.5000	0.38	0.8150	0.62	2.263	1.73	0.2180	0.17
M 9 m.												
O 5 f.					0.7350	0.54			5.751	4.26	0.4980	0.37
M 10 m.	0.0264	0.019	0.0386	0.028	0.5298	0.38	0.7820	0.56	3.537	2.54	0.3494	0.25
M 1 m.	0.0196	0.012	0.0238	0.014	0.6992	0.41			5.264	3.19	0.3644	0.22
M 2 m.	0.0248	0.015	0.0338	0.020	0.6690	0.40	1.3420	0.80	5.419	3.24	0.4190	0.25
S 26 m.	0.0402	0.024	0.0186	0.011	0.6410	0.38	1.0220	0.60	2.931	1.71	0.1630	0.10
S 16 f.	0.0392	0.021	0.0616	0.033	0.8306	0.45	1.0130	0.55	7.641	4.11	0.4390	0.24
S 25 m.	0.0928	0.047	0.0219	0.011	0.7850	0.40	1.1240	0.57	3.914	1.97	0.2670	0.14
S 27 m.	0.0486	0.022	0.0230	0.011	0.7980	0.37	1.0900	0.50	8.228	3.75	0.3790	0.17
M 13 m.												
S 28 m.												
Average (acute)	0.0380	0.023	0.0294	0.020	0.6687	0.43	0.9680	0.61	4.587	2.88	0.3177	0.21
M 3 m.	0.0202	0.017	0.0190	0.016	0.6762	0.55	0.5262	0.43	6.297	5.16	0.3714	0.30
M 12 m.	0.0250	0.020	0.0268	0.021	0.4756	0.38	0.6680	0.53	4.321	3.46	0.3650	0.29
M 5 m.	0.0158	0.013	0.0308	0.024	0.5510	0.43			3.046	2.40	0.1802	0.14
M 4 m.	0.0174	0.014	0.0168	0.013	0.4942	0.38	0.7460	0.58	7.039	5.45	0.4170	0.32
M 6 m.	0.0400	0.030	0.0462	0.034	0.5710	0.43	0.8820	0.66	5.958	4.44	0.6184	0.46
M 11 m.	0.0410	0.026	0.0256	0.016	0.5780	0.36	0.8940	0.56	4.654	2.98	0.4818	0.30
Average (chronic)	0.0266	0.020	0.0275	0.021	0.5577	0.42	0.7430	0.55	5.219	3.98	0.4056	0.30

TABLE 3—*Continued.*

RAT NO. AND SEX	STOMACH-INTESTINES (FILLED)		STOMACH-INTESTINES (EMPTY)		SUPRARENALS		KIDNEYS		TESTIS		EPIDIDYMI		HYPOPHYSIS	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	8.360	7.15	4.530	3.88	0.0468	0.040	1.075	0.92	(0.0600)*	(0.051)*				
M 8 m.	6.655	5.16	3.585	2.78	0.0380	0.029	1.374	1.07	1.754	1.36	0.4666	0.36	0.0066	0.0051
O 6 f.	8.270	6.38	4.680	3.60	0.0330	0.025	1.374	1.06						
M 7 m.	6.509	4.96	3.729	2.84	0.0414	0.032	1.235	0.94	1.675	1.28	0.4860	0.37	0.0060	p.0046
M 9 m.														
O 5 f.	8.720	6.46	7.250	5.37	0.0420	0.031	1.484	1.10						
M 10 m.	6.860	4.94	4.290	3.08	0.0344	0.025	1.410	1.01	2.179	1.57	0.5590	0.43	0.0068	0.0049
M 1 m.	6.090	3.69	3.280	1.99	0.0414	0.025	1.513	0.92	1.502	0.91	0.4490	0.27	0.0070	0.0043
M 2 m.	7.040	4.22	4.290	2.57	0.0338	0.020	1.505	0.90	1.124	0.90	0.4680	0.67	0.0066	0.0040
S 26 m.	7.100	4.15	4.550	2.66	0.0398	0.023	1.557	0.91	1.905	1.11	0.7640	0.45	0.0076	0.0045
S 16 f.	11.980	6.44	8.610	4.63	0.0416	0.022	1.634	0.88	(0.0616)*	(0.033)*			0.0146	0.0078
S 25 m.	8.540	4.31	5.070	2.56	0.0458	0.023	1.929	0.97	1.479	0.75	0.4120	0.21	0.0048	0.0024
S 27 m.	10.320	4.71	6.270	2.86	0.0424	0.019	2.150	0.98	2.430	1.11	0.7210	0.33	0.0096	0.0044
M 13 m.														
S 28 m.														
Average (acute)	8.040	5.21	5.010	3.25	m0.0396 f0.0409	0.025 0.030	1.520	0.97	1.756	1.12	0.5407	0.39	m0.0069 f0.0146	0.0043 0.0078
M 3 m.	10.480	8.60	6.530	5.40	0.0252	0.021	1.252	1.04	0.682	0.56	0.3060	0.25	0.0058	0.0048
M 12 m.	7.060	5.70	3.660	2.90	0.0306	0.025	1.418	1.13	1.829	1.46	0.4400	0.35	0.0052	0.0042
M 5 m.	6.030	4.80	3.660	2.90	0.0320	0.025	1.153	0.91	1.201	0.95	0.3910	0.31	0.0058	0.0046
M 4 m.	8.950	6.90	4.220	3.30	0.0496	0.038	1.465	1.13	1.113	0.86	0.3430	0.27	0.0062	0.0048
M 6 m.	8.480	6.30	4.580	3.40	0.0270	0.020	1.235	0.92	1.708	1.27	0.6160	0.46	0.0060	0.0045
M 11 m.	9.080	5.70	4.580	2.90	0.0326	0.021	1.379	0.87	1.597	1.00	0.5410	0.34	0.0064	0.0040
Average (chronic)	8.35	6.30	4.540	3.50	0.0328	0.025	1.317	1.00	1.355	1.02	0.4400	0.33	0.0059	0.0045

*Ovaries.

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CHANGES IN THE RELATIVE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS OF YOUNG ALBINO RATS HELD AT CONSTANT BODY-WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

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When young, actively growing animals are held at constant body-weight by underfeeding for considerable periods, one of three results might be expected *a priori* concerning the weights of the various organs and parts. (1) Since the body-weight remains constant, the weights of the individual organs and parts might also remain constant. (2) Since the normal growth rates are known to vary in the different organs and parts, one might expect those organs with the strongest normal growth tendency to increase in weight at the expense of the remainder of the body. (3) Since, however, the food-supply available is insufficient for both maintenance and growth, the animals are actually in a condition of chronic inanition; and changes might be expected to occur corresponding to those which have been observed in adults during inanition.

A few observations have been recorded upon changes in young animals in certain organs under these conditions, notably by Waters ('08), Aron ('11 and '14) and Donaldson ('11). A more extended and complete analysis of the changes in the organism under these conditions seemed highly desirable, and therefore the present investigation was undertaken. The work was begun at the University of Missouri, and continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. This grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc. An abstract of the present paper has been published (Jackson '15 b).

MATERIAL AND METHODS

The albino rat (Mus norvegicus albinus) was chosen for use in this experiment. It is a convenient animal on account of its comparatively small size, rapid growth and hardiness under experimental conditions. It is also practically the only mammal whose growth norm (including variability) for the whole body and for the various parts, systems and organs throughout the post-natal life cycle is even approximately known. The effects of inanition upon the adult rat have also been worked out (Jackson '15 a, '15 c) and are valuable for comparison.

The material used in the present experiment included ten litters (and one individual from an eleventh litter) as shown in table 1.

TABLE 1

Litters used in experiments

LITTER NO.	SOURCE	NO. AND SEX OF EACH LITTER	NO. TEST ANIMALS	CONSTANT WEIGHT PERIOD	NO. CONTROL ANIMALS	KILLED AT AGE OF
L 4. . .	Missouri	1 M. 4 F.	1 M. 3 F	3–6 wks.	1 F.	6 wks.
L 2. . .		3 M. 2 F.	1 M. 1 F.	6–32 wks.	2 M. 1 F.	32 wks.
L 3. . .		4 M. 2 F.	2 M. 1 F.	10–35 wks.	1 M. 2 F.	35 wks.
S 5. . .	Minneapolis	3 M. 8 F.	1 M. 1 F.	3–8 wks.	1 M. 1 F.	3 wks.
			5 F.	3–10 wks.	1 M. 1 F.	10 wks.
S 6. . .		2 M. 4 F.	1 M. 3 F.	3–10 wks.	1 M. 1 F.	10 wks.
S 7. . .		4 M. 2 F.	3 M. 1 F.	3–10 wks.	1 M. 1 F.	3 wks.
S 11. .		2 M. 4 F.	1 M. 2 F.	3–10 wks.	1 M. 1 F.	3 wks.
					1 F.	10 wks.
S 12. .		5 M. 3 F.	3 M. 3 F.	3–10 wks.	1 M.	3 wks.
					1 M.	10 wks.
S 10. .		1 M. 2 F.	1 F.	3–16 wks.	1 M. 1 F.	3 wks.
28. . . .		4 M. 4 F.	2 M. 2 F.	3-6 wks.	1 M. 1 F.	3 wks.
			1 F.	3–8 wks.	1 M.	6 wks.
22. . . .		1 F.	1 F.	3–13 wks.		
Total		29 M. 36 F.	15 M. 25 F.		13 M. 12 F.	

As noted in this table, three of the litters are from the rat colony at the University of Missouri, and the remainder from a local colony at the University of Minnesota. There are included twenty-nine males and thirty-six females, a total of sixty-five. In addition, two rats not listed in tables 1 and 2 were used as additional controls at three weeks in the study of the skeleton (table 7).

In most cases, the experiment began when the rats were three weeks of age (time of weaning). These rats were held at con-

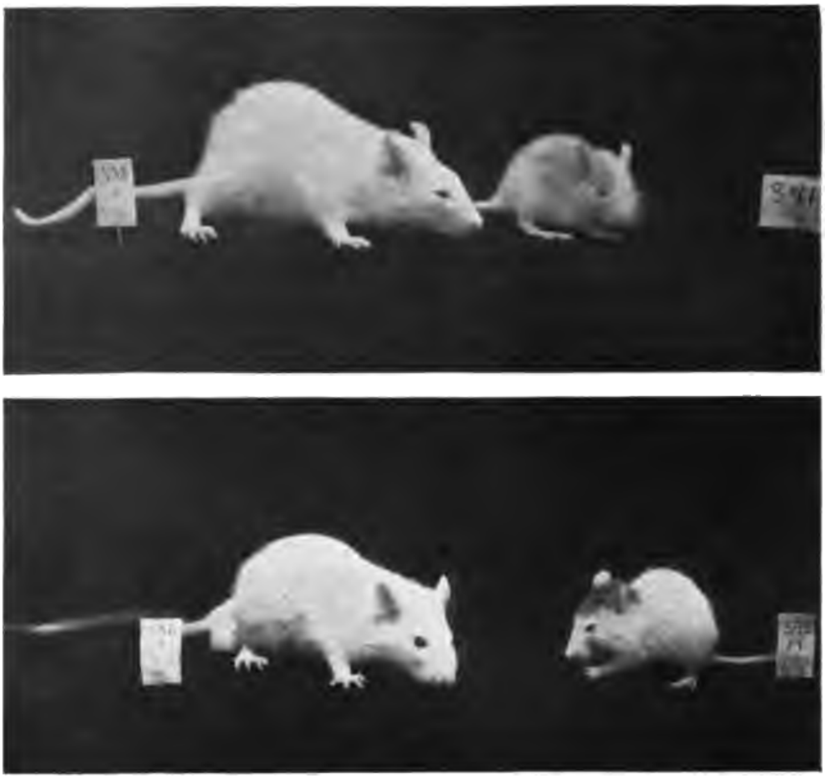

Fig. 1 Photograph showing four albino rats at the age of ten weeks. The larger rats represent the normal, full-fed controls. The smaller rats are from the same litter, but have been held by underfeeding at constant body-weight since the age of three weeks. The rats are pure albinos (Mus norvegicus albinus) the dark spot on the head of one being an artificial mark of identification.

stant body-weight for varying periods, from the age of three weeks up to the age of six weeks (8 rats) to eight weeks (3), to ten weeks (22), to thirteen weeks (1) and to sixteen weeks (1). A few were held at constant body-weight beginning at later periods,—from age of six weeks to age of thirty-two weeks (2), and from age of ten weeks to age of thirty-six weeks (3). Controls (25 in all) were also killed at the beginning and at the end of these time periods. At ten weeks of age, the normal controls are half-grown rats, sexually mature, of nearly adult proportions, and five or six times as heavy as those held at constant body-weight from the age of three weeks (fig. 1). In addition, a large number of observations upon the normal rat previously published (by Donaldson, Hatai, Jackson, Lowrey and others) are available for comparison. Of the animals used in this experiment, the distribution of sexes is given in table 1.

In table 2, the net body-weight (gross body-weight, including intestinal contents, is slightly higher), of the animals used is indicated. At each time period the average weight (and range) for each sex is given, for both controls and test animals held at constant body-weight. The cards containing the original individual records for all animals used will be deposited in The Wistar Institute of Anatomy, where they may be consulted if desired.

The rats were kept in ordinary wire cages (with wire-net bottoms, allowing the feces to drop through) and were individually weighed daily before feeding. Those under experiment

TABLE 2

Net body-weight of rats when killed; average weight and range indicated

CONTROLS (FULL-FED)			EXPERIMENTED (BODY-WEIGHT HELD CONSTANT)		
Controls killed at	No. and sex	Weight and range: grams	Weight and range: grams	No. and sex	Test animals constant for
3 weeks..	6 M.	24.4 (19.0-32.6)			
	5 F.	24.7 (21.1-30.4)			
6 weeks..	1 M.	42.3	22.4 (20.5-23.6)	3 M.	3-6 weeks
	1 F.	42.4	22.0 (20.6-23.8)	5 F.	
8 weeks			18.1	1 M.	3-8 weeks
			21.2 (17.8-24.6)	2 F.	
10 weeks	3 M.	155 (141-167)	24.7 (20.7-31.4)	8 M.	3-10 weeks
	3 F.	114 (109-118)	23.3 (18.5-31.0)	14 F.	
			25.5	1 F.	3-13 weeks
			26.0	1 F.	3-16 weeks
32 weeks.	2 M.	209 (203-215)	43.7	1 M.	6-32 weeks
	1 F.	166	50.4	1 F.	
35 weeks	1 M.	238	79.6 (73.6-85.5)	2 M.	10-35 weeks
	2 F.	158 (153-162)	74.2	1 F.	
Total	13 M. 12 F			15 M. 25 F.	

were fed an amount just sufficient to hold them constant at the initial body-weight. Of course slight fluctuations in the gross weight were unavoidable, but they rarely exceeded one gram above or below the initial weight. The food used was in all cases whole wheat (Graham) bread soaked in whole milk. Previous experience has shown that, at least up to the age of one year, albino rats thrive and develop normally upon this simple diet. Water *ad libitum* was also supplied.

It is a curious fact that under these circumstances the amount of food necessary for maintenance of body-weight apparently decreases as the experiment proceeds. Thus rats of about 25 grams gross body-weight when three weeks of age at the beginning of the experiment will at ordinary room temperature require about 5 grams of milk-soaked bread daily for maintenance. Later, this will usually decrease to an average of about 3 grams toward the age of ten weeks.[1] This is the opposite of what might be expected: (1) because at later periods the amount of available food supply stored in the body has been greatly diminished; and (2) because the animals held at constant body-weight almost invariably become much more active, requiring a greater expenditure of energy. Possibly the smaller amount of food required to maintain the animals at the later periods may be due to a greater absorption of water, thus maintaining a body-weight which would otherwise decline with the given amount of food. It is well known that during inanition in general the percentage of water-content of the body increases, and it is quite probable that during chronic inanition resulting from maintenance of a young, growing animal at constant body-weight the amount of living protoplasm in the body decreases. If the amount of metabolism is thereby decreased, a smaller food-supply would suffice for maintenance.

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[1] Two examples may be cited. Six rats of litter No. 12 were held at constant body-weight (within a range of 1 gram) from the age of three weeks on June 21, 1914, average body-weight 23.6 grams, for seven weeks to the age of ten weeks on August 6, 1914, at which time the average gross body-weight was 23.8 grams. The average daily food-supply of whole wheat (Graham) bread soaked in whole milk for the seven consecutive weeks of the experiment was as follows: 5.1, 3.9, 3.7, 3.5, 3.3, 2.7, 2.7 grams. Similarly, six rats of litter No. 13, average weight 23.1 grams at three weeks of age on June 28, 1914, were held at constant weight for seven weeks until August 12, 1914, when at ten weeks of age their average gross weight was 22.6 grams. Their average daily food-supply for the seven consecutive weeks was as follows: 5.3, 5.0, 4.1, 3.9, 3.3, 3.2, 2.9 grams. In all cases water (city supply, from the Mississippi river) was supplied *ad libitum*. The diminishing amount of food necessary for maintenance cannot be explained as due to increasing temperature, as this was fairly constant. Moreover, a similar condition has been found in other litters at all seasons of the year.

As will be shown later, on account of the intensity of the growth-impulse, especially during the earlier periods of inanition, certain growth-changes occur which require the expenditure of energy. It is possible that this energy is supplied by the excess of food above that required for maintenance proper. Another, but less probable, explanation might be that during inanition the food-intake is in some way more economically utilized, a smaller quantity therefore being sufficient for maintenance. In the later stages of inanition, there is probably a decrease in the temperature of the body, which would therefore require less food.

Rats held at constant body-weight from the age of three weeks to ten weeks, while becoming more active as the experiment proceeds, become at the same time less resistant to cold. They may die suddenly if the room temperature is lowered, or even without any apparent cause. Thus up to sixteen weeks, the longest successful period in those beginning at three weeks, it becomes increasingly difficult to maintain them alive at constant body-weight. When the experiment is begun later, the length of the time during which the body-weight can be held constant is considerably increased. Aron ('11) had a similar experience with dogs, finding it necessary after a time to feed sufficiently to increase the initial body-weight somewhat, in order to keep the animals alive. He explains this as due to the gradual exhaustion of available food-substance stored in the various tissues of the body.

At the end of the various age-periods of the experiment, and at the beginning and end for controls, the rats were killed by chloroform and dissected according to the technique described in previous papers (Jackson and Lowrey '12; Jackson '13, '15 c). The parts, systems and organs were carefully weighed, and portions preserved for microscopic examination (to be considered in a later paper).

As heretofore, in calculating the percentage weights, the *net* body-weight (gross weight less intestinal contents) is taken. The percentage weights of the organs are thus slightly higher than if calculated upon the gross body-weight.

The averages given in the various tables are the arithmetical means of the corresponding individual observations. In view of the comparatively small number of observations and the known variability, especially of some of the organs (cf. Jackson '13), the data are insufficient for treatment by statistical methods, and the values are therefore only fair approximations. They are, however, sufficiently accurate to show some of the more obvious and important changes in the young animal held at constant body-weight. It is hoped that they may be useful as preliminary observations, which may lead to further and more extensive investigations of the various individual organs. In general, the amount of variation found is sufficient to necessitate great caution in drawing conclusions from a small number of observations (sometimes upon a single animal), as frequently happens in experimental work.

LENGTHS OF BODY AND TAIL

The body-length is measured from the tip of the nose to the anus, and the tail-length from the anus to the tip of the tail. The measurements were taken immediately after death, the body and tail being extended by very slight tension. Measurements during life are not practicable, although they might be obtained by the use of anesthetics.

In order therefore to discern the changes in the lengths of body and tail while the body-weight is held constant, it was necessary first to determine these measurements on the normal animal. For this purpose, 450 observations (267 males, 183 females) were available, varying from newborn to about 400 grams body-weight. Of these, 277 (130 males, 147 females) were from the Missouri rats described in a previous paper (Jackson '13), and 25 (13 males, 12 females) from Minnesota. For the remaining 148 (124 males, 24 females) observations upon rats from the colony at The Wistar Institute in Philadelphia, I am indebted to Professor Donaldson and Dr. Hatai. A careful examination revealed no essential differences in the relations of body and tail-lengths according to the source of the rats, so they were all combined into a single series.

The general relations of body and tail-lengths are evident from table 3 (not including the Wistar data, in which the age was usually unknown).

The average ratio of tail to body-length in table 3 was obtained by calculating the ratio for each individual separately, and then taking the mean of the individual ratios. The results are thereby somewhat more accurate than would be obtained by simply taking the ratios of the *average* tail and body-lengths, though the difference is not great.

TABLE 3

Changes in lengths of body and tail

AGE	NUMBER OF OBSERVATIONS		AVERAGE NET BODY-WEIGHT: GRAMS		AVERAGE BODY-LENGTH: MM.		AVERAGE TAIL-LENGTH: MM.		AVERAGE RATIO OF TAIL TO BODY-LENGTH		
	male	female	male	female	male	female	male	female	male	female	total
a. Normal rats (full-fed)											
Newborn	32	36	4.8	4.6	48.7	45.5	16.9	17.5	0.35	0.37	0.36
1 week........	23	27	9.6	9.7	64.5	63.3	29.4	31.4	0.46	0.50	0.48
3 weeks.......	22	22	21.7	18.7	90.4	86.1	59.1	57.9	0.65	0.67	0.66
6 weeks......	21	16	47.4	45.4	128.0	124.0	110.0	110.0	0.86	0.90	0.88
10 weeks......	19	15	128.0	99.4	173.0	160.0	150.0	143.0	0.87	0.89	0.88
5 to 13 mo.....	16	34	177.7	148.0	190.0	183.0	162.0	168.0	0.86	0.90	0.88
b. Rats under experiment (body-weight held constant)											
(from age of)											
3 to 6 weeks...	3	4	22.4	22.1	98.0	94.4	72.3	77.4	0.74	0.82	0.78
3 to 8 weeks...	1	2	18.1	21.2	94.5	92.0	78.0	80.0	0.83	0.87	0.84
3 to 10 weeks.	8	14	24.7	23.3	105.2	99.6	86.2	83.9	0.82	0.85	0.84
3 to 13 weeks.	0	1		25.5		91.0		85.0		0.93	0.93
3 to 16 weeks.	0	1		26.0		90.0		95.0		1.06	1.06
6 to 32 weeks.	1	1	43.7	50.5	115.0	115.0	105.0	105.0	0.91	0.91	0.91
10 to 35 weeks	2	1	79.6	74.2	140.0	135.0	117.0	115.0	0.84	0.85	0.85

Two facts concerning the relative length of the tail in the normal albino rat are apparent from table 3 a. In the first place, it is evident that at all ages the ratio of the tail-length to the body-length is slightly greater in the female than in the male; that is, the female is relatively long-tailed. By a comparison of the ratios, it appears that the tail of the female on the average is about 4 or 5 per cent longer.

In the second place, it appears that the ratio of tail-length to body-length increases in the normal rat from an average of about 0.36 at birth to 0.48 at one week, 0.66 at three weeks, and 0.88 from the age of six weeks upward. That is, the tail becomes progressively relatively longer, being relatively more than twice as long in the adult as at birth. The acceleration of the tail-growth at such a later period may be cited as an instance of the general law of cranio-caudal progression in development (Jackson '09).

Turning now to the tail-ratio in the rats held at constant body-weight, as shown in table 3 b, and also indicated in the chart in figure 2, it is clear that in rats beginning at three weeks of age there has been a very decided increase in the tail-ratio. The tail at this age evidently continues to elongate, even though the body-weight has been held constant, so that the tail-ratio approaches (although it does not usually quite reach) the normal ratio for rats of corresponding age under normal conditions of growth.

If we compare the absolute lengths of tail and body in the normal rat at three weeks (table 3 a) with those in rats held at constant body-weight from the age of three weeks to the age of six, eight, ten, thirteen and sixteen weeks, it appears that there has also been a slight increase in the absolute length of the body. The increase in tail-length is considerably greater, however, so the tail-ratio increases as above stated.

The larger number of rats were held constant from the age of three to the age of ten weeks. Since the average body-weight for the normal series at three weeks is slightly lower than that of the series held constant to ten weeks of age, I have obtained a new normal series of higher body-weight for direct comparison

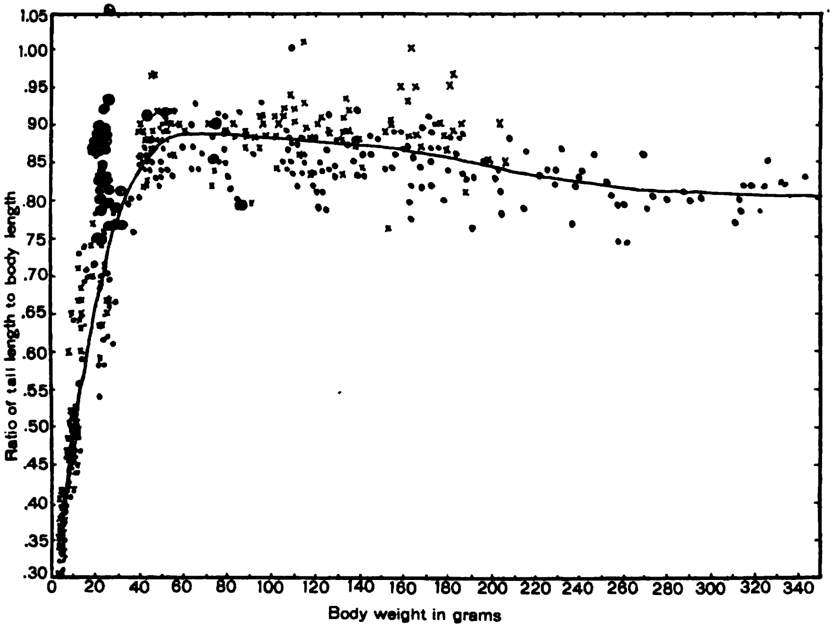

Fig. 2 Chart showing the change in the ratio of tail-length to body (nose-anus) length in the albino rat from birth to maturity. Males are indicated by rounded dots, females by X. The individuals held at constant body-weight during the experiment are encircled. The curve is drawn through points representing the means for the normal individuals at various periods, sexes combined.

(by dropping out the lighter rats of the series given in table 3 a). Thus in sixteen normal males aged three weeks, average net body-weight is 24.2 grams, the average body-length is 94.5 mm. and tail-length is 61.6 mm. (compared with 105.2 mm. body-length and 86.2 mm. tail-length in the 3 to 10 weeks experiment). Similarly, in eight normal females at three weeks, average net body-weight 23.2 grams, the average body-length is 91.1 mm., and tail-length is 63.4 mm. (compared with 99.6 mm. body-length and 83.9 mm. tail-length in the 3 to 10 weeks experiment). In other words, while the body has increased about one-tenth in length, the tail has increased over one-third, the body-weight being held constant from the age of three to ten weeks.

In order to show more clearly this change in the ratio of tail-length to body-length, the individual ratios corresponding to the 450 observations on rats from all sources (including the 148 Wistar rats) were plotted according to body-weight in figure 2. As the sexes are distinguished in the entries, it is evident that the females tend to have a higher tail-ratio. The curve has been drawn through the averages at various periods (sexes combined). It is smoothed free hand, as the labor of constructing the curve more accurately by mathematical methods did not seem justified.

Special attention is called to one apparent discrepancy between the curve in figure 2 and the data in table 3 a. In the latter, it appears that the tail reaches at six weeks an apparent maximum ratio of 0.88 (sexes combined), which is maintained nearly constant at succeeding periods. In the chart, however, it is seen that in rats above 300 grams the average ratio drops to nearly 0.80. These heavy rats are nearly all from the Wistar series, and are all males. It is therefore evident that the drop in the tail-ratio curve is in part due to the fact that no females are included in the higher body-weights. Even taking this into account, however, there is still a decrease in the tail-ratio of the male from a maximum of about 0.86 or 0.87 in rats between 50 and 200 grams body-weight, to nearly 0.80 in rats above 250 grams. (In 7 male rats between 350 and 400 grams, not shown in figure 2, the ratios were slightly above the 0.80 line, the average being 0.81).[2]

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[2] Since the completion of the present paper, I have received, through the courtesy of Professor Donaldson, a manuscript copy of reference tables compiled at The Wistar Institute by formulas for various measurements of the albino rat. These include the body-lengths and tail-lengths, by sexes, from newborn to adult. From these data I have calculated the tail-ratios and find the result in general agreement with the curve shown in figure 2. The tail-ratios calculated from the Wistar tables are somewhat lower, corresponding to body-weights from 30 to 100 grams, however. They also increase steadily, so that at body-weights above 200 grams they lie slightly above the curve in figure 2. The tail-ratio according to the Wistar tables is about 4 per cent higher in the female throughout, when the sexes of equal body-length or body-weight are compared. In rats above 300 grams body-weight, the tail-ratio is about 0.86 in the male and 0.89 in the female.

The two observations on rats held constant from the age of three weeks to the ages of thirteen and sixteen weeks would seem to indicate a continuation of the process of elongation of the tail, even beyond the normal ratio at corresponding age, but the number of observations is too small for definite conclusion.

The number of animals beginning the experiment at later ages, body-weight constant from age of six to age of thirty-two weeks (2) and from ten to thirty-five weeks (3), is also too small to draw any very positive conclusions. However, so far as they go, they indicate (table 3 b; fig. 2) that *between the ages of six and thirty-five weeks there is no marked change in the tail-ratio of rats held at constant body-weight.*

It will be observed that also in the normal rats between six and thirty-five weeks of age there is no apparent change in the tail-ratio, whereas between three and six weeks of age there is normally a decided increase in the tail-ratio. The lengths of the body and tail are of course determined primarily by the growth of the skeleton. I would therefore interpret the results concerning the lengths of tail and body as follows. In young, growing rats held at constant body-weight, the body and tail tend to increase so as to assume the normal ratio at corresponding ages. This is due to the fact that, as will appear later, the skeleton continues to grow in a normal manner (though at a reduced rate) in animals held at constant body-weight.

There is another possible factor in causing the increased tail-ratio in young, growing rats, which may also apply to the similar relative elongation of the tail found in adult rats (cf. Jackson '15 c). Professor Donaldson points out (in a personal communication) that during inanition there may be an arching of the spinal column, producing an actual shortening of the body-length. Such an arching actually does occur, and may be noted especially in young rats during chronic inanition. It is well shown in the stunted rats photographed in figure 1. Of course the greater part of this longitudinal curvature of the spinal column is eliminated by the slight tension exerted in order to straighten out the body when it is measured after death. But it is still quite possible that this does not entirely eliminate the shortening of the column. In any event, however, this is probably a factor of minor importance in altering the tail-ratio during inanition.

Hatai ('08) in a series of five 'stunted' rats in which growth had been retarded (but not stopped) by a diet of starch-mixtures from age of 30 days up to from 127 to 215 days (the final body-weight being from 70.9 to 113.7 grams) finds the average tail-ratio 0.75 as compared with about 0.82 in controls. He notes that:

> The most conspicuous external differences between normal and stunted rats as shown by the stunted rats are in the length of the body and of the tail, both of which were considerably reduced with respect to the body-weight. This peculiar difference, as is seen from the table, holds true in every case. Further, the ratio between the length of the body and that of the tail is considerably less in the stunted rats than in the control rats. . . . Underfeeding therefore produces short tailed individuals.

Recently, however, Dr. Hatai (in a personal communication) states that in other inanition experiments he has obtained different results, and that "rats either grown or kept in a state of chronic inanition (starch feeding, lipoid-free ration and wheat embryo feeding) give a longer tail" in agreement with my results.

Morgulis ('11) in the salamander Diemyctylus found a relatively greater shrinkage in the tail than in the body during inanition; while Harms ('09) found the converse to be true in Triton.

HEAD

The head (table 4; fig. 3) at three weeks normally forms an average of 22.5 per cent of the body, the average net body-weight being 21.2 grams. In the 11 controls at three weeks, the body-weight (24.6 grams) is slightly higher, and the corresponding relative head-weight, 20.6 per cent, somewhat lower. In the rats held constant from the age of three weeks to the ages of six and eight weeks, the average percentage of the head (21.6 per cent and 23.9 per cent) is higher than that of the controls. But the average body-weight in these groups is lower, more nearly the normal above cited, so it is doubtful whether there is any actual increase in the head-weight (either relative or absolute) during the experiment. The larger ten weeks group, however, is nearly equal to the controls in average body-weight, and shows an apparent increase in head-weight from an average of 5.01 to 5.34 grams, or from 20.6 per cent to 22.7 per cent (table 4; fig. 3). In any event, however, the increase in the head-weight is slight, and is not apparent in the two rats held constant from three to thirteen and sixteen weeks (average of the two is 20.6 per cent). On the other hand, there appears to be a slight increase in the weight of the head in the rats held constant from six to thirty-two weeks (15.2 to 17.7 per cent), and from ten to thirty-five (12 to 14.0 per cent).

On the whole, therefore, the evidence would appear to indicate that in young rats held at constant body-weight for considerable periods of time there is a slight increase in the weight of the head. This is probably due to the increase in skeletal weight, which in the head probably overbalances the decrease in the weight of the

TABLE 4

The head; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal head at 3 weeks (Jackson '13)	24	21.2	4.60 (3.44– 5.85)	22.5 (16.3–27.3)
Controls at 3 weeks	11	24.5	5.01 (4.70– 5.90)	20.6 (18.3–23.0)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.4	4.83 (4.50– 5.10)	21.6 (19.9–23.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	4.73 (4.60– 4.90)	23.9 (19.9–26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	5.34 (4.60– 6.50)	22.7 (20.4–26.2)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	5.00	19.6
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	5.50	21.6
Normal at 6 weeks (Jackson '13)	42	50.0	7.40 (6.30–9.40)	15.2 (11.3–18.0)
Controls at 6 weeks	2	42.4	6.40 (6.20– 6.60)	15.0 (14.6–15.5)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	8.30 (8.20– 8.50)	17.7 (16.8–18.6)
Controls at 10 weeks	6	134.0	13.30 (11.10–14.70)	10.1 (8.6–11.7)
Body-weight constant 25 weeks (age of 10 to 35 weeks (normal head for corresponding body-weight of 85 grams forms about 12.0 per cent	1	85.5	12.10	14.0
Controls at 32 and 35 weeks	6	189.5	20.30 (17.40–26.30)	10.7 (9.8–11.4)

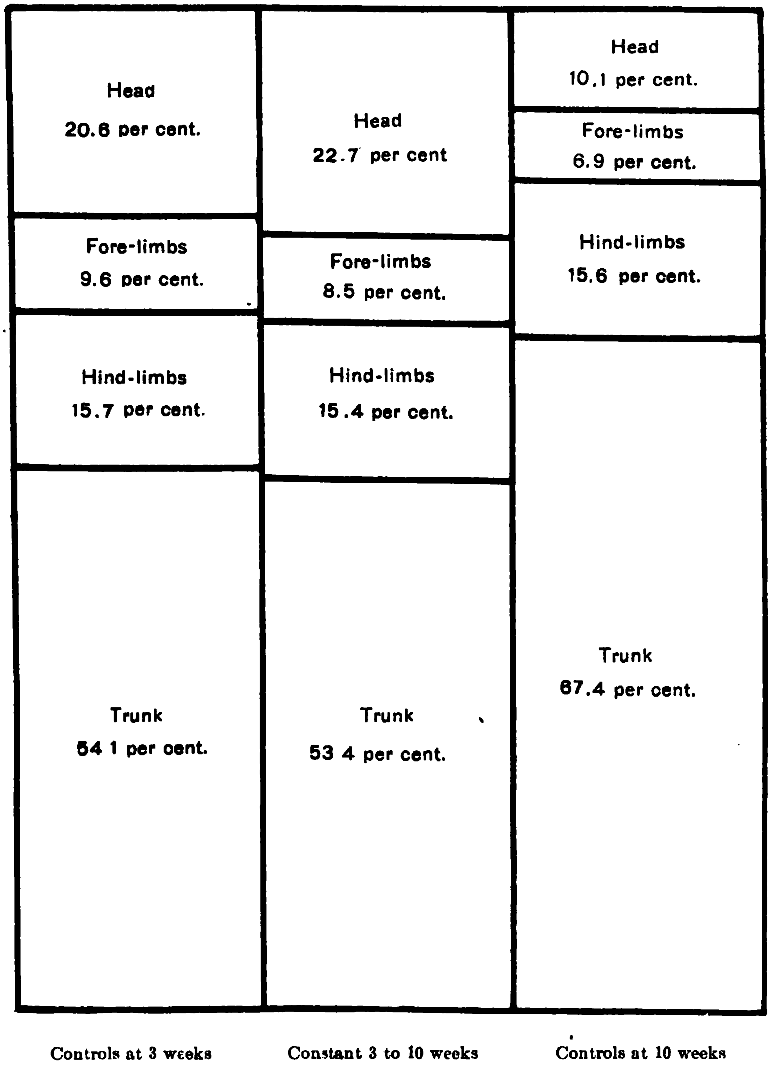

Fig. 3 Diagram representing the average relative (percentage) weights of parts of the body (head, extremities and trunk) in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

integument; although, as will be shown later, the average loss in the integument of the *entire body* is relatively slightly greater than the corresponding skeletal increase (fig. 4).

In both acute and chronic inanition in adult rats (Jackson '15 a, '15 c) the head increases very markedly in relative weight, the loss in absolute weight being but very slight in comparison with the loss in weight of the entire body.

EXTREMITIES AND TRUNK

The extremities (table 5; fig. 3) were separated at the shoulder-joint and hip-joint, respectively. There is apparently a slight decrease in the relative weight of the fore-limbs in the young rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks of age. In the case of the rats held constant from three to ten weeks, the apparent decrease is from an average of 9.6 per cent to 8.5 per cent. On account of the small number of observations, however, and the difficulty in separating the limbs (especially the integument) in an absolutely uniform manner, the slight apparent decrease is of doubtful significance.

In the case of the hind-limbs, there is likewise an apparent indication of a slight decrease, but even less marked than in the fore-limbs. The apparent average decrease from 15.7 per cent to 15.4 per cent of the body-weight in the largest group (three to ten weeks, is well within the limits of experimental error.

On the whole, therefore, it is doubtful whether there is any distinct and significant change in the weights of the extremities in young rats held at constant body-weight for considerable periods. A slight loss, however, might be accounted for by the slightly greater loss in the integument (as compared with the gain by the skeleton); especially since the integument of the limbs probably forms a relatively larger part of the limbs than the whole integument does of the whole body.

The trunk was not weighed directly, but its weight was calculated by subtracting from the net body-weight the weight of the head and extremities. From what has been said con-

TABLE 5

The extremities; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
a. Fore-limbs				
Normal at 3 weeks (Jackson and Lowrey)	4	25.5	2.36	9.3 (7.4–10.90)
Controls at 3 weeks	6	23.8	2.29 (2.00–2.51)	9.6 (8.7–10.60)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	23.1	1.68 (1.50–1.90)	7.3 (6.7– 8.30)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	1.70	6.9
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.3	1.84 (1.60–2.20)	8.5 (7.4- 9.50)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	1.80	7.1
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	1.80	6.9
Normal at 6 weeks (Jackson and Lowrey)	4	79.2	5.30	6.7 (5.9– 8 22)
Controls at 6 weeks	1	42.3	3.00	7.1
Controls at 10 weeks	2	127.9	8.90 (7.20–10.60)	6.9 (6.3– 7.60)
Normal at 10 weeks (Jackson and Lowrey)	3	141.9	7.60	5.3 (5.21–5 50)
b. Hind-limbs				
Normal at 3 weeks (Jackson and Lowrey)	4	25.5	3.80	14.9 (14.3–15.40)
Controls at 3 weeks	6	23.8	3.75 (3.20–4.13)	15.7 (14.3–15.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	23.1	2.97 (2.55–3.60)	12.8 (11.6–15.10)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	3.40	13 8
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.3	3.38 (3.00–3.80)	15.4 (14.3–15 90)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	3.20	12.4
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.20	12.6
Normal at 6 weeks (Jackson and Lowrey)	4	79.2	11.80	14.9 (14 6-15.40)
Controls at 6 weeks	1	42.3	5.60	13.2
Controls at 10 weeks	2	127.9	19.90	15.6 (15.4–15 80)
Normal at 10 weeks (Jackson and Lowrey)	3	141.9	22.10	15.6 (14.5–16 50)

TABLE 6

The integument; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal integument at 3 weeks (Jackson and Lowrey)	13	24.8	5.55	22.4 (18.7 –29.2)
Controls at 3 weeks	10	25.1	5.59 (3.86– 6.36)	21.9 (18.4 –25.2)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	2.81 (2.21– 3.21)	12.5 (9.82–14.1)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	2.78 (2.27– 3.30)	13.8 (12.6 –15.5)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	3.41 (2.63– 4.51)	14.5 (12.3 –17.5)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	3.50	13.7
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.30	12.7
Normal at 6 weeks (Jackson and Lowrey)	14	64.4	13.46	20.9 (16.7 –25.9)
Controls at 6 weeks	2	42.4	8.04 (8.00– 8.08)	17.0 (15.1 –19.0)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	6.15 (5.70– 6.60)	13.0 (12.9 –13.1)
Normal at 10 weeks (Jackson and Lowrey)	10	131.0	24.50	18.7 (15.6 –22.3)
Controls at 10 weeks	6	134.0	26.70 (19.40–32.10)	20.0 (17.8 –22.0)
Bodyweight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	14 00 (12.30–17.20)	17.9 (16.6 –20.1)
Controls at 32 and 35 weeks	6	189.5	38.40 (28.10–55.20)	20.1 (18.0 –23 2)

cerning the head and extremities, it follows that there cannot be much change in the trunk-weight, since the probable slight increase in the weight of the head is off-set by a slight decrease in the extremities. In the largest group, however, held at constant body-weight from three to ten weeks of age (fig. 3), the trunk would apparently decrease from an average of 54.1 per cent to 53.4 per cent. This apparent change is so slight as to be (probably) insignificant.

The results concerning the parts of the body therefore fail to indicate any decided change of proportional weights in young animals held for considerable periods at constant body-weight. There is apparently a very small increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to be of doubtful significance. During inanition in adult rats, there is apparently a relative increase in both head and extremities, counterbalanced by a relative decrease in the trunk (Jackson '15 a, ' 15c).

INTEGUMENT

In the rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks, there is a very marked loss in the weight of the integument (including hair and nails; table 6; fig. 4). In the case of the largest (three to ten weeks) group, the decrease is from an average of 21.9 per cent to 14.5 per cent of the body-weight. In terms of absolute weight, the decrease is from 5.30 grams (5.59 grams, less correction on account of difference in body-weight, which averages 25.1 grams at three weeks and 23.8 grams at ten weeks) to 3.41 grams, a decrease of about 36 per cent. The decrease would appear slightly greater if the difference in relative weight of the integument for different initial body-weights were taken into account. There is apparently even greater loss at the other ages. It would appear that this loss (which is perhaps chiefly a loss of fat) occurs rather early, as at six weeks (body-weight held constant three weeks) the loss is as great as at subsequent and longer periods.

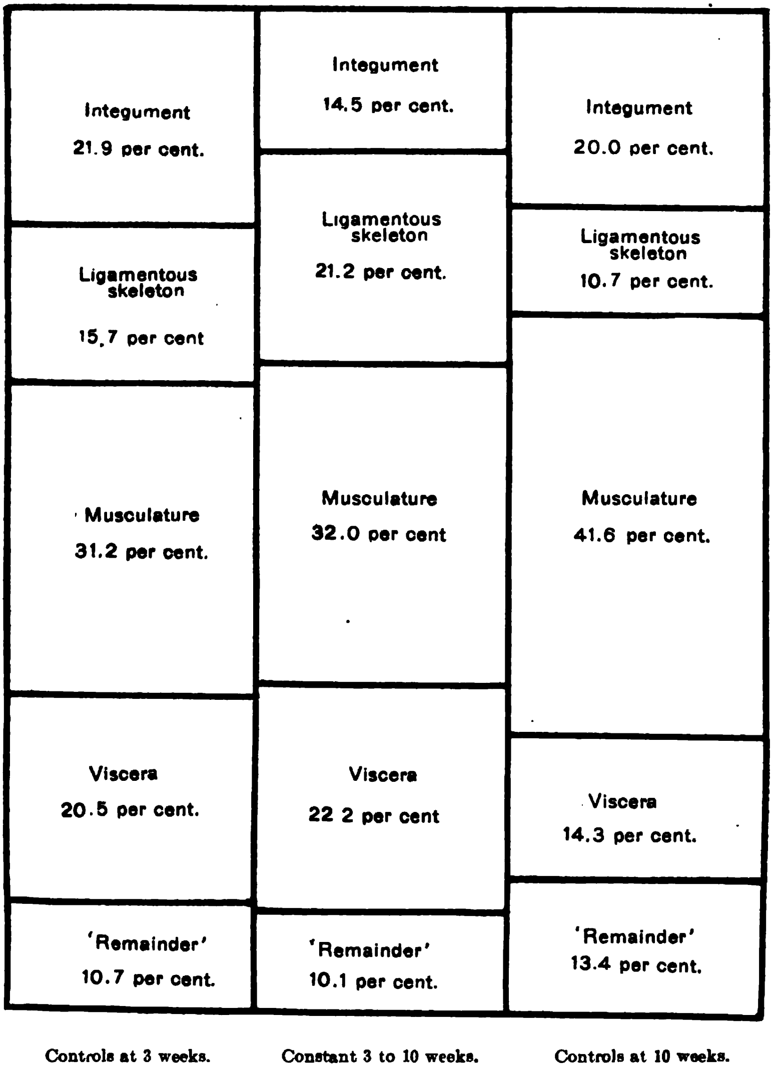

Fig. 4 Diagram representing the average relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

In the rats experimented upon at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) there is also a marked loss in the weight of the skin, though apparently not so great as at the earlier periods.

This loss in the weight of the integument is in striking contrast with the results of inanition in adult rats (Jackson '15 a, '15c). Here the loss is very nearly proportional to that of the whole body, so the integument nearly maintains its relative (percentage) weight.

From his experiments upon young dogs held at constant body-weight, Aron ('11, p. 29) states that: "The skin shows a slightly higher percentage of the body-weight in those animals kept at a constant weight than in the normal, control dogs. These figures indicate that, while the (body) weight was constant, the skin increased very slightly in weight." The figures cited show the skin in animals held at nearly constant body-weight to form (in four cases) 12.2 to 14.6 per cent of the body-weight, whereas in three corresponding full-fed controls the skin formed 11.2 to 13.0 per cent. Aron, however, overlooks the fact that he is making his comparison with controls at the *end* of the experiment. In order to judge what changes have taken placed during the experiment, the comparison must be with normal control animals killed at the *beginning* of the experiment. Aron records but one case which can be used for this purpose. His Dog D (table 13, Experiment IV) killed at the age of 40 days, the beginning of the experiment, with body-weight of 1985 grams shows a skin-weight of 320 grams, or about 16.1 per cent of the body-weight. From Aron's own data, therefore, I would reach the opposite conclusion, viz., that in young dogs held at constant body-weight, the skin suffers a marked loss in weight. This would agree with my results on rats.

SKELETON

The skeleton (table 7; fig. 4) was prepared in three ways. The bones, together with the cartilages, periosteum and ligaments, constitute the 'ligamentous skeleton' (table 7 a). The bones and cartilages, after removal of the periosteum and ligaments by immersion for about one hour in 1 per cent aqueous ‘gold dust ’solution at 90°C., constitute the ‘cartilaginous skeleton’ (table 7 b). Finally the cartilaginous skeleton dried in an oven at 95°C. to constant weight constitutes the ‘dry skeleton’ (table 7 c).

An examination of the weight of the ligamentous skeleton (table 7 a) reveals the striking fact that while the body-weight

TABLE 7

The skeleton; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
a. Ligamentous skeleton				
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	4.12	16.6 (13.1 -21.10)
Controls at 3 weeks	12	24.5	3.90 (3.100- 5.46)	15.7 (13.5 -17.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	4.04 (3.140- 5.50)	18.0 (14.6 -23.90)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	4.74 (4.290- 5.50)	23.7 (22.3 -24.90)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	4.98 (3.700- 7.14)	21.2 (17.7 -24.60)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	4.80	18.8
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	5.20	20.0
Normal at 6 weeks (Jackson and Lowrey)	14	64.4	9.02	14.0 (10.5 -20.10)
Controls at 6 weeks	2	42.4	5.48 (4.750- 6.20)	13.0 (11.2 -14.70)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	7.20 (6.800- 7.60)	15.4 (15.1 -15.60)
Normal at 10 weeks (Jackson and Lowrey)	10	131.0	15.30	11.7 (10.0 -12.90)
Controls at 10 weeks	6	134.0	14.00 (11.400-17.60)	10.7 (8.5 -14.10)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	10.30 (9.400-11.60)	13.3 (12.7 -13.70)
Controls at 32 and 35 weeks	6	189.5	18.60 (15.600-24.30)	9.8 (8.8 -11.20)
b. Cartilaginous skeleton (Fresh)				
Controls at 3 weeks	6	22.9	2.60 (2.120- 3.00)	11.4 (9.0 -12.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	22.9	3.56 (2.930- 4.04)	15.5 (13.3 -17.00)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	4.50	18.3
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.4	3.16 (2.420- 4.00)	14.6 (11.9 -17.10)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	4.00	15.7
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.96	15.2
Controls at 10 weeks	1	115.0	6.40	5.6
c. Dry skeleton (cartilaginous)				
Controls at 3 weeks	5	23.4	0.804 (0.710-0.964)	3.43 (3.17- 4.03)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	21.9	1.091 (1.033-1.172)	4.98 (4.70- 5.13)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	1.351	4.59
Body-weight constant 7 weeks (age of 3 to 10 weeks)	9	22.2	1.285 (1.076-1.485)	5.84 (4.76- 7.00)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	1.068	6.31
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	1.744	6.71
Control at 10 weeks	1	115.0	3.420	2.97

is held constant the skeleton continues to increase in weight to a marked degree. In the rats beginning at three weeks, there is an increase in the relative weight of the ligamentous skeleton from 15.7 per cent of the body to 18.0 per cent at six weeks and to an apparent maximum of 23.7 per cent at eight weeks. This latter is probably an exceptional figure, as in the largest group, at ten weeks, the average is 21.2 per cent (fig. 4). This corresponds to an increase from an absolute weight of 3.90 to 4.98 grams, an increase of about 28 per cent (or slightly more, if correction be made for the difference in body-weight, average 24.5 grams at three weeks and 23.8 grams at ten weeks). The two cases carried to thirteen and sixteen weeks, respectively, show a slightly smaller relative increase. The rats used at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) also show a considerable increase in the skeleton, though relatively less than those beginning at the earlier period.

The data for the cartilaginous skeleton (table 7 b) similarly show a marked increase in rats held at constant body-weight for various periods beginning at three weeks of age. The figures for the largest group (three to ten weeks) indicate an increase from 11.4 per cent to 14.6 per cent of the body. In terms of absolute weight, the increase is from an average of 2.60 grams (body-weight 22.9 grams) to 3.16 grams (body-weight 22.4 grams), an increase of about one-fourth. Subtracting the percentage weights of the cartilaginous skeleton from the corresponding ligamentous skeleton, there is (for the three to ten weeks group) an evident increase of the ligaments and periosteum from 4.3 per cent to 6.6 per cent of the net body-weight. This would indicate that the ligamentous component of the skeleton shares in the marked growth during constant body-weight.

Professor Donaldson (in a personal communication) has kindly supplied a series of observations showing that the cartilaginous skeleton in the normal rat changes from a relative weight of about 10 per cent of the body at 20 grams to 7.5 per cent at 50 grams, 7 per cent at 100 grams and 6.7 per cent in rats above

200 grams. These weights, however, do not include the intervertebral discs.

The data for the dried cartilaginous skeleton (table 7 c) indicate an even greater increase in the dry skeleton of the rats held at constant body-weight. Thus in rats beginning at three weeks the dry substance increases from 3.43 per cent of the body weight to 4.98 per cent at six weeks of age, 5.49 per cent at eight weeks, 5.84 per cent at ten weeks, 6.31 per cent at thirteen weeks and 6.71 per cent at sixteen weeks.

Since the increase in the dry skeleton is relatively greater than that for the (moist) cartilaginous skeleton, it necessarily follows that the skeleton must be losing in percentage of water and gaining in percentage of dry substance. The percentage of dry substance has been calculated for each individual skeleton included in tables 7 a and 7 b, and the averages for each group are as follows: controls at three weeks, 31.4 per cent; constant three to six weeks of age, 33.5 per cent; three to eight weeks, 30.0 per cent; three to ten weeks, 41.7 per cent; three to thirteen weeks, 40.2 per cent; three to sixteen weeks, 44.0 per cent; control at ten weeks, 53.4 per cent.

Lowrey ('13) finds the dry substance of the *ligamentous* skeleton in the normal albino rat to increase from an average of 33.3 per cent at 20 days of age to 39.2 per cent at six weeks, 45.9 per cent at ten weeks, 50.4 per cent at five months and 52.6 per cent at one year.

From the foregoing it is evident that in rats held at constant body-weight beginning at three weeks, the growing cartilaginous skeleton steadily increases its percentage of dry substance. Thus it tends to change the proportions of water and dry substance as during normal growth. The percentage of dry substance does not increase so rapidly with age as during normal growth, however, but lags behind corresponding to the retardation in absolute growth. During inanition in adult rats, on the contrary, there is a relative decrease in the dry substance, and an increase in water-content (Jackson '15 c).

It has already been noted in a previous section ("Lengths of body and tail") that in the rats held at constant body-weight beginning at the age of three weeks there is an increase in the lengths of both body and tail. The latter increases more rapidly. however, so that it tends to assume the tail-ratio found in normal rats of corresponding age. This indicates that the skeleton not only continues to grow (though at a reduced rate) while the body-weight is held constant, but also tends to *grow in a normal manner*, so as to produce the normal ratio of tail-length and body-length. The preceding paragraphs have shown that the increased growth of skeleton affects the ligamentous as well as the cartilaginous and bony components, and that the chemical composition (percentages of water and dry substance) also changes in a manner tending to assume the normal.

The question naturally arises as to whether the skeletal growth during constant body-weight is merely a growth in mass, or is associated with the normal process of *differentiation*. During the present investigation a few observations have been made upon the development of the normal skeleton, indicating some of the more obvious changes during the age-periods of the rats under experiment, especially between the ages of three and ten weeks. While a detailed study of the developmental changes in the skeleton is reserved for a separate paper, some preliminary conclusions may be noted here.

In skeletons of rats held at constant body-weight from the age of three to the age of ten weeks, the appearance and fusion of certain epiphyses may be noted as in the normal animal during this period, although in most cases the process appears to be retarded somewhat. The following examples may be cited. In the normal skeleton at three weeks of age, the epiphyses at the ends of the vertebral bodies have not appeared; the epiphysis at the lower end of the humerus is well developed, but not fused with the shaft; the maxilla and mandible present each two molars (on each side), with no visible trace of a third. In the normal skeleton at ten weeks, the epiphyses at the ends of the vertebral bodies have appeared, and most of them have united with the corresponding bones; the epiphysis at the lower end of the humerus is firmly fused with the shaft; well developed third molar teeth have appeared, both in the maxilla and in the mandible. In the skeleton of a rat held at constant body-weight from three to ten weeks of age, most of the epiphyses of the vertebral bodies have appeared, and they usually have united at one end of each bone; the lower epiphysis of the humerus is firmly united with the shaft, as normally at ten weeks; well-developed third molars have appeared, as normally at ten weeks. In a rat held at constant body-weight from age of three to sixteen weeks, the skeletal differentiation was more advanced, corresponding at least to the stage reached normally at ten weeks, and in some respects perhaps even beyond it.

These observations will suffice to establish the fact that the skeletal growth during constant body-weight is accompanied by normal developmental changes, as well as changes in chemical composition (percentage of water). In other words, we find not only increase in mass but growth and differentiation apparently normal in character, though somewhat retarded in rate. These skeletal characters therefore tend to correlation with age, although influenced also by the general body-weight.

The remarkable fact that the skeleton continues to grow while the body-weight is held constant was apparently first observed by Waters ('08) who found that calves previously well nourished will continue to increase in height and in width of hip for a considerable time, even when increase of body-weight is prevented by under-feeding. He remarks ('08 b, p. 9):

> Apparently the animal organism is capable of drawing upon its reserve for the purposes of sustaining the growth process, for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a certain extent to support growth, the process ceases and there is no further increase in height or in length of bone. From this point on, the animal's chief business seems to be to sustain life. This law applies to animals on a stationary live weight as well as to those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance, and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, get thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.

Aron ('11) experimented with dogs to determine the effect of a restricted amount of food upon young, growing animals. He found that in spite of constant live weight, the animals continued to increase in length and height for three to five months. Thereupon the emaciated animals became weaker and died unless the amount of food was somewhat increased. From a comparative study of nine bones (the entire skeleton was not measured), Aron concludes that the skeleton during constant body-weight increases in mass and also changes in chemical composition (increase in water-content and protein (?); decrease in fat). The results of this very interesting investigation, while sufficient to establish the continued growth of the skeleton, would be more conclusive if the number of observations were larger, with an adequate number of controls at the beginning and at the end of the experiment. In a recent paper, Aron ('14) records a few observations indicating that malnutrition in children retards growth in length less than body-weight; so that the body may continue to increase in length while the body-weight is at a standstill, or even slightly decreasing. Thus the strong growth tendency of the skeleton during bare maintenance of the body-weight is manifest in the human species, as well as in the calves, dogs and rats.

MUSCULATURE

Although the musculature (table 8; fig. 4) in the normal rat at three weeks averages 26.9 per cent of the body, according to Jackson and Lowrey ('12), the controls in the present series gave a somewhat higher amount, the average being 31.2 per cent. As shown in table 8, the musculature in the controls of the present series also averaged slightly higher than the normal according to Jackson and Lowrey at six and ten weeks. In rats held at constant body-weight from the age of three weeks to six, ten and thirteen weeks, the musculature appears relatively very slightly higher than in the controls at three weeks. The apparent increase from three to ten weeks is from 7.40 grams (7.81 grams, less correction corresponding to the smaller body-weight at 10 weeks) to 7.62 grams, or an increase of 3.0 per cent in absolute weight. In the three to sixteen weeks experiment,

TABLE 8

The musculature; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	6.67	26.9 (20.1-30.2)
Controls at 3 weeks	10	25.1	7.81 (6.90-9.82)	31.2 (29.5-35.3)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	7.04 (5.51-8.05)	31.8 (25.1-34.7)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	6.46 (5.68-7.70)	32.1 (31.3-33.2)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	7.62 (4.58-10.87)	32.0 (24.8-36.4)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	7.90	31.0
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	7.50	29.4
Normal at 6 weeks (Jackson and Lowrey)	14	64.4	21.10	32.7 (26.1-35.3)
Controls at 6 weeks	2	42.4	14.90 (14.85-15.0)	35.3 (35.0-35.6)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	16.90 (15.30-18.5)	35.7 (34.8-36.6)
Normal at 10 weeks (Jackson and Lowrey)	10	134.0	55.10	41.1 (37.4-49.1)
Controls at 10 weeks	6	135.0	55.80 (44.00-69.2)	41.6 (39.3-44.5)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	31.20 (30.10-33.9)	40.1 (39.7-40.7)
Controls at 32 and 35 weeks	6	189.5	81.20 (64.80-119.7)	42.6 (39.0-50.3)

TABLE 9

Viscera and remainder; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	RELATIVE WEIGHT OF VISCERA (AND RANGE) PER CENT	RELATIVE WEIGHT OF 'REMAINDER' (AND RANGE) PER CENT
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	21.3 (20.1-24.8)	12.9 (4.0-19.9)
Controls at 3 weeks	10	25.1	20.5 (17.9-24.8)	10.5 (2.6-15.6)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	25.0 (20.7-29.2)	13.6 (7.1-21.6)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	26.8 (25.0-28.5)	2.3 (1.9-2.7)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	22.2 (19.4-26.0)	10.0 (2.5-16.9)
Normal at 6 weeks (Jackson and Lowrey)	14	64.5	20.4 (18.4-22.9)	12.0 (6.5-17.1)
Controls at 6 weeks	1	42.4	19.6	19.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	19.5 (19.1-19.9)	16.4 (16.1-16.7)
Normal at 10 weeks (Jackson and Lowrey)	10	130.5	16.0 (14.9-17.2)	12.5 (1.2-18.4)
Controls at 10 weeks	6	135.0	14.3 (13.0-15.7)	13.6 (9.7-16.4)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	16.3 (16.1-16.8)	12.4 (9.7-13.9)
Controls at 32 and 35 weeks	5	179.8	12.9 (11.9-13.5)	17.0 (13.7-19.5)

the average is slightly lower. In the six to thirty-two weeks experiment, the musculature appears very slightly higher than in the controls at six weeks, while in the ten to thirty-five weeks experiment, the musculature appears slightly lower than in the controls at ten weeks. In the latter case, however, the controls are too heavy for comparison with those under experiment.

In general, it seems clear from the foregoing that in young rats held at constant body-weight the musculature also remains nearly constant in weight, with perhaps a very slight tendency to increase in the majority of cases. In the course of normal growth during this period, the musculature shows a more rapid growth than any other system, increasing from about 27 per cent of the body at three weeks to 41 per cent at ten weeks of age (Jackson and Lowrey). During inanition in adult rats, the musculature loses approximately in proportion to the entire body, slightly less in acute inanition and slightly more in chronic inanition (Jackson '15 c)

Aron ('11) did not weigh the muscles in his experiments on dogs, but infers (p. 29) that: "Only the flesh, muscles and fat of the body remain as the tissues which must have lost during the course of the experiments." From an analysis of samples taken from the leg muscles, he also concludes that "The muscles contained only one-half of the normal amount of solids," the protein being greatly decreased and the water-content increased. Again, however, his comparison is with controls at the *end* rather than the *beginning* of the experiment, so that no conclusion can be drawn as to the changes taking place during the experiment in the animals held at constant body-weight.

VISCERA AND 'REMAINDER'

With the visceral group (table 9; fig. 4) have been included the brain, spinal cord and eyeballs, as well as the thoracic and abdominal viscera. According to Jackson and Lowrey '12, this group decreases from about 21 per cent of the body at three weeks to about 16 per cent at ten weeks of age. This is in fairly close agreement with the controls in the present series, except at ten weeks. In this case the controls are considerably too heavy for direct comparison with the animals under experiment, which accounts for the discrepancy.

In the animals held at constant body-weight from the age of three weeks to the ages of six, eight and ten weeks, the visceral group shows a distinct increase in weight. This is more marked at six and eight than at ten weeks, which perhaps indicates that the viscera may increase in the earlier part of the experiment, and lose weight later. The experiment from six to thirty-two weeks indicates no essential change in the weight of the viscera. From ten to thirty-five weeks there is a slight gain.

On the whole, it may be concluded that during constant body-weight in young albino rats the visceral group as a whole undergoes but little change in weight, with a slight tendency to increase, especially in the earlier periods. As will be seen later, however, the individual viscera differ greatly in their reactions.

Aron ('11) concludes that in young dogs held at nearly constant body-weight the organs in general do not lose weight. On account of the small number of observations, however, and the lack of adequate controls, it is difficult to draw any satisfactory conclusion from his observations upon the viscera.

The 'remainder' is obtained by deducting from the net body-weight the weight of the integument, skeleton, musculature and viscera. It therefore includes loss by evaporation and escape of fluids, as well as a few small unweighed organs and the masses of dissectable fat. The data in table 9 show a considerable variation, as might be expected. On the whole, however, it appears doubtful whether there is any material change in the weight of the 'remainder' in young rats held at constant body-weight for considerable periods. There is undoubtedly a loss in the fat, but this is probably counterbalanced by an increased water-content of the interstitial connective tissues.

BRAIN

The brain (table 10) in eleven controls at three weeks of age averaged 1.282 grams, or 5.31 per cent of the (net) body-weight. This corresponds fairly closely with Donaldson's ('08) figure for the normal rat of corresponding weight. In the rats held at constant body-weight from the age of three to six and eight weeks, there appears (table 10) to be a relative increase in the brain, especially at eight weeks, where it forms 6.62 per cent of the body. In reality, however, this relative increase is only apparent, and due to the fact that these animals began the experiment at a lower body-weight, corresponding to which the brain is relatively heavier. The (net) body-weights of the two rats at eight weeks were respectively 18.1 grams and 17.8 grams. According to Jackson ('13, p. 22), the brain normally reaches its maximum relative weight of about 6.7 per cent of the body when the body-weight is about 15 grams. Thus the final brain-weight of the rats held at constant body-weight from the age of three to eight weeks of age is almost exactly that to be expected if the brain-weight has remained constant.

TABLE 10

The brain; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1)	52	25.0*	1.285	5.14
Controls at 3 weeks	11	24.5	1.282 (1.187–1.364)	5.31 (4.14–6.20)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.195 (1.035–1.297)	5.44 (4.50–6.33)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	1.183 (1.180–1.186)	6.62 (6.60–6.63)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.267 (1.136–1.379)	5.30 (4.38–6.20)
Normal at 6 weeks (Donaldson '08)	42	45.0*	1.441	3.20
Control at 6 weeks	1	42.4	1.373	3.23
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	1.478 (1.459–1.497)	3.14 (2.96–3.32)
Normal at 10 weeks (Donaldson '08)	34	75.0*	1.559	2.08
Controls at 10 weeks†	6	134.0	1.579 (1.512–1.636)	1.21 (0.96–1.44)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	1.646 (1.603–1.723)	2.12 (2.02–2.18)
Controls at 32 and 35 weeks	6	189.5	1.777 (1.657–1.890)	0.97 (0.78–1.24)

* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

The brain weight has also apparently remained nearly constant in the large group held at constant body-weight from three to ten weeks of age. The average absolute weight is slightly less, but almost in correspondence with the body-weight, so that the average *relative* weight, 5.30 per cent, is nearly identical with that of the controls at three weeks, the beginning of the experiment. In terms of absolute weight, there is a very slight apparent decrease from 1.274 grams (1.282 grams, less correction[3] for difference in body-weight, which averages 24.5 grams in the three weeks controls and 24.0 grams at ten weeks) to 1.267 grams, a decrease of about 0.5 per cent in absolute weight.

In the series held at constant body-weight from the age of six to thirty-two weeks, there is an apparent slight decrease in the brain from about 3.23 per cent to 3.14 per cent of the body, and in the ten to thirty-five weeks series a slight increase (from 2.08 to 2.12 per cent). Considering the small number of observations and the normal variation, however, these apparent differences do not appear to be significant. I would, therefore, conclude from the data above cited that there is probably no appreciable change in the weight of the brain in young albino rats held at constant body-weight for considerable periods.

Hatai ('04) experimented with a series of young rats with initial body-weights corresponding roughly to those of mine at the ages of six to ten weeks. By giving an unfavorable diet (starch and beef-fat) their body-weight was reduced on the average about 30 per cent. The brain in these cases had apparently lost in absolute weight, the average loss being about 5 per cent. These results, however, are of course not directly comparable with those in which the body-weight has remained constant.

In a later experiment, Hatai ('08) by underfeeding with unfavorable diet retarded the growth of a series of five rats, beginning at the age of 30 days, so that at 170 days their average weight was only 91.5 grams, while full-fed controls averaged 146.5 grams. By comparison with 'second controls,' younger rats of body-weight similar to the final weight of the stunted series, he found that in the stunted rats the brain-weight was practically identical with that of normal rats of the same body-weight. In other words, the growth in brain-weight had been retarded in the same proportion as the body-weight. On this principle, if the body-weight were retarded so as to permit no growth at all, that is held at constant weight, we should expect practically no increase in weight of the brain. This is in agreement with my results, as above stated.

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[3] It should be noted here as in other cases that the correction for organ-weight is not in exact proportion to the difference in body-weight. Allowance must be made for the change in the *relative* weight of the organ corresponding to the change in body-weight.

More recently Donaldson ('11) has experimented with a larger series (twenty-two litters) of rats held at nearly constant weight (34 grams) from the age of thirty to the age of fifty-one days. In the rats held at constant body-weight, the brain weight averaged 7.7 per cent less than in full-fed controls of the same litters. No direct controls were taken at the *beginning* of the experiment, but from the normal growth formula it is estimated that the initial brain-weight was slightly less than that found in the retarded rats at the end of the experiment. This would indicate an increase of 3.6 per cent in the brain-weight, while the body-weight was held constant. The large number of observations lends weight to this conclusion, although it would be strengthened if direct controls were available at the beginning of the experiment.

It may be noted that if Donaldson's normal (Wistar reference tables) rather than the direct controls be taken as the basis for estimating the initial brain-weight in my three to ten weeks series, the result would indicate a gain similar to that found by Donaldson in his series. On the whole, therefore, we may safely conclude that there is but very slight if any increase in the brain-weight of young albino rats held at constant body-weight for considerable periods of time.

SPINAL CORD

When the relative weights are compared with the controls at the beginning of the experiment (table 11), or with the theoretical normal according to Donaldson ('08) there appears a very decided increase in the spinal cord at all the age-periods during the experiment. Thus while the body-weight has been held constant from the age of three to that of ten weeks, the spinal cord has apparently increased from an average of 0.179 to 0.243 grams, an increase of about 36 per cent (or slightly more if the initial weight be decreased to correct for the difference of body-weight at three weeks, 24.5 grams, and ten weeks, 24.0 grams). This corresponds to an increase from 0.74 per cent to 1.02 per cent of the net body-weight. The increases at the other age-periods are equally striking.

Donaldson ('11) in the experiments previously mentioned also found an increase in the weight of the spinal cord in rats held at body-weight of about 34 grams from the age of thirty days to that of fifty-one days. He does not estimate this increase exactly but from the normal weight of the cord at the beginning of the experiment (cf. Donaldson '08, table 1) the weight must have increased from about 0.223 to 0.2498 grams, an increase of about 10.7 per cent. While this is not so striking as my results (perhaps in part because my experiments covered a longer period of time) it agrees in indicating during constant body-weight a much stronger growth tendency in the spinal cord than in the brain. This is in agreement with the well-known fact that in general the normal post-natal growth of the spinal cord is relatively much more rapid than that of the brain. This growth of the spinal cord is apparently correlated with the increase in trunk-length (Donaldson).

EYEBALLS

An increase even more striking than that of the spinal cord is apparent in the eyeballs (table 12). In rats held at constant body-weight from the age of three weeks, the eyeballs increase from a relative weight of about 0.50 per cent of the body-weight to 0.64 per cent at six weeks, 0.82 per cent at eight weeks, and

TABLE 11

The spinal cord; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1)	47	25.0*	0.180	0.72
Controls at 3 weeks	11	24.5	0.179 (0.152–0.200)	0.74 (0.56–0.89)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.0	0.207 (0.149–0.235)	0.96 (0.63–1.25)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.192 (0.188–0.195)	1.08 (1.06–1.09)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.243 (0.201–0.314)	1.02 (0.89–1.17)
Normal at 6 weeks (Donaldson '08)	42	45.0*	0.254	0.57
Controls at 6 weeks	1	42.4	0.286	0.67
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.399 (0.378–0.420)	0.85 (0.83–0.86)
Normal at 10 weeks (Donaldson '08)	32	75.0*	0.333	0.44
Controls at 10 weeks†	6	134.0	0.422 (0.341–0.464)	0.32 (0.27–0.39)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.520 (0.500–0.550)	0.67 (0.64–0.69)
Controls at 32 and 35 weeks	6	189.5	0.603 (0.491–0.667)	0.33 (0.24–0.44)

* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

TABLE 12

The eyeballs; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	24	21.2	0.105 (0.073–0.125)	0.52 (0.31–0.73)
Controls at 3 weeks	10	24.6	0.120 (0.110–0.133)	0.50 (0.34–0.69)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.1	0.142 (0.138–0.149)	0.64 (0.60–0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.146 (0.145–0.146)	0.82 (0.81–0.82)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.179 (0.130–0.196)	0.76 (0.57–1.00)
Normal at 6 weeks (Jackson '13)	42	50.0	0.153 (0.125–0.175)	0.32 (0.18–0.40)
Control at 6 weeks	1	42.4	0.152	0.36
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.240 (0.230–0.250)	0.51 (0.50–0.52)
Normal at 10 weeks (Jackson '13)		75.0	0.173	0.23
Controls at 10 weeks*	6	134.0	0.209 (0.195–0.228)	0.16 (0.13–0.21)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.275 (0.249–0.323)	0.35 (0.34–0.38)
Controls at 32 and 35 weeks	5	184.4	0.260 (0.235–0.296)	0.15 (0.12–0.17)

* Body-weight of controls at 10 weeks too high for comparison.

0.76 per cent at ten weeks. In terms of absolute weight, the eyeballs have apparently increased from an average of 0.120 grams at three weeks to about 0.179 grams (no correction made for the slight difference in body-weight) at ten weeks, an increase of nearly 50 per cent! In the normal, full-fed rat at ten weeks (average body-weight 112 grams) the eyeballs have reached a weight of only about 0.201 grams (Jackson '13). At this rate, the weight of the eyeballs at a normal body-weight of 75 grams (the body-weight indicated in table 12 as 'normal at 10 weeks.' to correspond to the body-weight of the animals held at constant body-weight from the age of ten to thirty-five weeks) would be only about 0.173 grams, or slightly less than that actually reached in the series held at constant body-weight of 24 grams from three weeks to ten weeks of age. The growth of the eyeballs in rats held constant from the age of six to thirty-two weeks, and from ten to thirty-five weeks, is equally striking.

No data upon the growth of the eyeballs under these conditions have been found in the literature. I have shown elsewhere (Jackson '15 a, '15 c), however, that the eyeballs lose but very little if any during inanition in the adult albino rat.

In connection with the astonishing growth capacity of the eyeballs in young animals at constant body-weight, the possibility that the growth of the eyeballs is somewhat independent of that in the body as a whole may be considered, which I have already pointed out (Jackson '13, p. 24). When the large water-content of the eyeballs is considered (85.6 per cent in the rat at twenty days, according to Lowrey '13), it is, after all, not difficult to comprehend the possibility of its continued growth, largely by water-absorption, when growth in the body as a whole is at a standstill.

THYROID GLAND

In young albino rats held at constant body-weight from the age of three weeks to six weeks, eight weeks and ten weeks, there is usually a well-marked loss of weight in the thyroid gland (table 13). In the largest group, three to ten weeks, the thyroid has apparently decreased on the average from about 0.033 per cent

TABLE 13

The thyroid gland; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	26	18.7	0.0060 (0.0034-0.0104	0.030 (0.018-0.041)
Controls at 3 weeks	11	24.6	0.0078 (0.0064-0.0094	0.033 (0.022-0.042)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	23.1	0.0053 (0.0052-0.0056	0.023 (0.022-0.024)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.0058 (0.0047-0.0068	0.032 (0.026-0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	18	24.1	0.0059 (0.0034-0.0087	0.025 (0.016-0.036)
Normal at 10 weeks (Jackson '13)	24	110.0	0.0165 (0.0100-0.030)	0.015 (0.008-0.022)

of the body to 0.025 per cent. Or, in terms of absolute weight, it has decreased from 0.0078 to 0.0059 grams, a decrease of 24 per cent. (A slight correction should be made on account of difference in body-weight.) No observations were made upon the thyroid gland in the experiments beginning at later ages.

During acute inanition in adult rats, the thyroid gland apparently loses little or no weight; while in chronic inanition with an average loss in body-weight of about 36 per cent, the thyroid gland loses only about 22 per cent in weight (Jackson '15). There is some uncertainty as to the exact figures, however, on account of variability and difficulty in dissecting out the thyroid gland in an accurate manner. The same, of course, holds true for the present series.

THYMUS

The normal thymus (table 14) at three weeks forms 0.37 per cent of the net body-weight. This decreases, in rats held at constant body-weight, to 0.075 per cent at six weeks of age, to 0.030 (exceptional?) at eight weeks, and to 0.040 per cent at ten weeks. In terms of absolute weight, the thymus has decreased from 0.091 grams at three weeks to 0.017 grams (loss of 81 per cent) at six weeks, and to 0.0094 grams (loss of 90 per cent) at 10 weeks. No correction for the slight difference in body-weight has been made in these estimates.

Normally at ten weeks of age the weight of the thymus should have increased to about 0.24 grams (0.30 grams in the controls).

A maximum absolute weight of about 0.29 grams is reached by the average normal thymus about the age of 85 days (Hatai '14). At one year, it has undergone a complete age-involution, and forms only 0.02 per cent of the body-weight (Jackson '13).

That the weight and structure of the thymus are markedly affected by various adverse conditions has long been known, and the process of involution has recently been thoroughly investigated by Hammar and his pupils. Jonson ('09) experimented with young rabbits subjected to acute and chronic inanition. In the latter case, the diet was restricted so as to maintain the young rabbits at constant body-weight (similar to the present experiment with rats). Under these conditions, Jonson found the weight-curve of the thymus similar to that of the body-fat, although during *acute* inanition the fat decreases somewhat more rapidly. In young rabbits held at constant body-weight the thymus in four weeks is reduced to about one-thirtieth of its initial weight. The cortex suffers the greatest loss, being reduced to one-twelfth of its initial weight within two weeks of chronic inanition at constant body-weight. It would therefore appear that the process of involution is much more rapid and complete in young rabbits than in young rats at the ages included in the present investigation. In both cases, however, the weight of the thymus in hunger involution decreases most rapidly in the earlier weeks of the experiment.

HEART

The heart (table 15) in the albino rats held at constant body-weight from the age of three weeks appears to have remained practically constant at about 0.70 per cent of the net body-weight up to the age of ten weeks. In absolute weight, the heart would apparently decrease from 0.167 grams (0.170 grams, less correction for difference in body-weight) to 0.166 grams, a decrease of about 0.6 per cent, which is probably within the limits of experimental error. The very slight decrease at six weeks and increase at eight weeks are also probably not significant. Similarly in the rats held at constant body-weight from six to thirty-two weeks and from ten to thirty-five weeks the heart has apparently retained almost exactly its initial relative weight. The absolute weight therefore apparently remains unchanged (the differences in the table being due to different initial body-weights).

During inanition in adult rats (Jackson '15 a, '15 c) the heart likewise maintains its relative weight, losing in absolute weight nearly in proportion to the entire body (slightly more in chronic than in acute inanition).

TABLE 14

The thymus; average absolute weight, average percentage weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGTH (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.071 (0.034 -0.135)	0.37 (0.23 -0.55)
Controls at 3 weeks	11	24.5	0.091 (0.042 -0.123)	0.37 (0.22 -0.51)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	22.7	0.017 (0.011 -0.022)	0.075 (0.046-0.0101
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.0054 (0.0037-0.0071)	0.030 (0.021-0.040)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.0094 (0.0054-0.0170)	0.040 (0.019-0.062)
Normal at 6 weeks (Jackson '13)	42	50.0	0.108 (0.052 -0.284)	0.21 (0.14 -0.36)
Normal at 10 weeks (Jackson '13)	42	107.2	0.24 (0.12 -0.44)	0.23 (0.13 -0.35)
Controls at 10 weeks	6	134.0	0.30 (0.25 -0.34)	0.23 (0.16 -0.29)

TABLE 15

The heart; average absolute weight; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.135 (0.082-0.250)	0.72 (0.56-0.93)
Controls at 3 weeks	11	24.5	0.170 (0.130-0.245)	0.70 (0.55-0.84)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.151 (0.123-0.187)	0.68 (0.63-0.85)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.129 (0.128-0.130)	0.72 (0.72-0.72)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.166 (0.124-0.213)	0.70 (0.55-0.83)
Normal at 6 weeks (Jackson '13)	42	50.0	0.277 (0.183-0.535)	0.55 (0.44-0.68)
Controls at 6 weeks	1	42.4	0.237	0.56
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.271 (0.255-0.288)	0.57 (0.57-0.58)
Normal at 10 weeks (Jackson '13)		75.0	0.375	0.50
Controls at 10 weeks*	6	134.0	0.562 (0.489-0.687)	0.44 (0.40-0.48)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.389 (0.348-0.450)	0.50 (0.47-0.52)
Controls at 32 and 35 weeks	6	189.5	0.873 (0.688-1.29)	0.46 (0.37-0.56)

* Body-weight of controls at 10 weeks too high for comparison.

LUNGS

The lungs (table 16) in rats held at constant body-weight from the age of three weeks to the ages of six weeks and ten weeks appear to lose in weight. (The data at eight weeks are abnormally high, as noted in the table). Thus between the ages of three weeks and ten weeks, the lungs apparently decrease from 1.04 per cent to 0.91 per cent of the body-weight; or in absolute weight from 0.250 to 0.218 grams, a loss of about 15 per cent. (A slight correction has been made on account of the difference in body-weight). In the few observations upon rats for longer periods beginning at the later ages of six and ten weeks, there appears to be no material change in the weights of the lungs during the experimental periods.

The lung infections frequently found in older and adult rats, rarely occur before the age of ten weeks, and thus do not affect the present series. As Hatai has already noted, rats during chronic inanition appear to be unusually free from lung infection.

During inanition in adult rats, the lungs lose weight in about the same proportion as the whole body, thus nearly maintaining their relative (percentage) weight. The loss is slightly greater, however, during chronic inanition (Jackson '15 c).

TABLE 16

The lungs; average absolute weight; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.216 (0.151–0.354)	1.17 (0.86–1.46)
Controls at 3 weeks	11	24.5	0.253 (0.201–0.290)	1.04 (0.89–1.12)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	20.9	0.192 (0.190–0.197)	0.92 (0.88–0.93)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.280* (0.241–0.319)	1.56* (1.36–1.77)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.218 (0.170–0.282)	0.91 (0.78–1.32)
Normal at 6 weeks (Jackson '13)	39	50.0	0.333 (0.244–0.547)	0.68 (0.58–0.94)
Control at 6 weeks	1	42.4	0.264	0.62
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.319 (0.309–0.329)	0.68 (0.65–0.70)
Normal at 10 weeks (Jackson '13)		75.0	0.49	0.65
Controls at 10 weeks	6	134.0	0.74 (0.610–0.94)	0.56 (0.45–0.74)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.49 (0.390–0.60)	0.63 (0.52–0.81)

* Lungs in one case abnormally heavy on account of post mortem congestion.

LIVER

The liver (table 17) in the albino rats held at constant body-weight from the age of three weeks to six, eight and ten weeks shows an apparent increase. This increase appears greatest in the shortest series, three to six weeks, increasing from 4.95 per cent of the body-weight (in controls) to 5.89 per cent. Between three and ten weeks the corresponding increase is from an absolute weight of 1.16 grams (1.20 grams, less correction for difference in body-weight; or 4.95 per cent of the body-weight) to 1.28 grams (5.25 per cent of the body-weight), or an increase of about 10.3 per cent in absolute weight.

In the later and longer periods, however, there appears to be a decided decrease in the weight of the liver (six to thirty-two weeks and ten to thirty-five weeks series). This may be due to the fact that the latter experiments extended over a longer period of time, or it may be because they were begun at a later age. We may therefore conclude that in young albino rats held at constant body-weight there is, beginning at three weeks, an increase in the weight of the liver (apparently greater at six than at ten weeks of age); while in rats beginning the experiment later, at six and ten weeks of age (and extending over a longer period)

TABLE 17

The liver; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.87 (0.42–2.08)	4.50 (3.21–5.82)
Controls at 3 weeks	11	24.5	1.20 (1.06–1.38)	4.95 (4.03–6.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.30 (0.89–1.63)	5.89 (4.32–7.39)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.92 (0.78–1.06)	5.12 (4.36–5.88)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.28 (0.80–1.82)	5.25 (3.63–7.00)
Normal at 6 weeks (Jackson '13)	42	50.0	3.19 (2.18–4.76)	6.48 (5.39–8.46)
Control at 6 weeks	1	42.4	2.18	5.15
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	2.33 (2.21–2.45)	4.44
Normal at 10 weeks (Jackson '13)		75.0	4.50	6.00
Controls at 10 weeks	6	134.0	6.72 (4.91–8.66)	4.99 (4.28–5.72)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	3.11 (2.30–3.51)	4.25 (3.91–4.74)
Controls at 32 and 35 weeks	6	189.5	7.27 (5.84–9.86)	3.84 (3.47–4.14)

there is a decided decrease in the weight of the liver. Thus the liver would appear under these conditions to have a tendency to follow the normal growth-impulse, which increases to a maximum at about the age of six weeks, and decreases thereafter (Jackson '13, p. 31).

During inanition in adult rats, the liver loses in weight relatively more than the whole body, and to a greater extent in acute than in chronic inanition (Jackson '15 c). As the liver is normally subject to great variation in weight, however, (Jackson '13) great caution must be observed in drawing final conclusions. Hatai ('13) found the weight of the normal liver distinctly higher than that in my series, and if his data instead of my controls were taken as a basis for the initial weights, the estimated losses in my experiments would be considerably greater, even the younger rats showing a loss instead of a gain in liver-weight.

SPLEEN

Taking the controls at three weeks as a basis for comparison, it appears that in rats held at constant body-weight from the age of three weeks to six weeks the weight of the spleen (table 18) remains practically unchanged (the average at six

TABLE 18

The spleen; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.055 (0.019–0.145)	0.28 (0.15–0.42)
Controls at 3 weeks	11	24.5	0.091 (0.051–0.130)	0.37 (0.27–0.48)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.087 (0.040–0.172)	0.38 (0.18–0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.043 (0.036–0.049)	0.24 (0.20–0.27)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.053 (0.033–0.076)	0.22 (0.16–0.33)
Normal at 6 weeks (Jackson '13)	42	50.0	0.135 (0.086–0.204)	0.28 (0.19–0.47)
Control at 6 weeks	1	42.4	0.107	0.25
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.139	0.29 (0.28–0.30)
Normal at 10 weeks (Jackson '13)		75.0	0.225	0.30
Controls at 10 weeks	6	134.0	0.350 (0.300–0.420)	0.27 (0.21–0.36)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.230 (0.230–0.240)	0.30 (0.27–0.33)
Controls at 32 and 35 weeks	6	189.5	0.620 (0.480–0.750)	0.33 (0.29–0.39)

weeks being probably too high, on account of the inclusion of a litter with abnormally large spleens); while in the rats at eight and ten weeks there is a considerable decrease. In the case of the three to ten weeks series, the decrease would be from 0.091 gram (0.37 per cent of the net body-weight) to 0.053 gram (0.22 per cent of the body-weight), or a decrease of nearly 42 per cent in absolute weight. (No correction has been made for the difference in body-weight, which is 0.5 gram lower at ten weeks). If the normal relative weight of the spleen at three weeks (0.28 per cent, Jackson '13) be taken as a basis of estimate, however, the loss would appear considerably less.

In the rats held at constant body-weight at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there appears to be no appreciable change in the average weight of the spleen. It may be concluded therefore that in young rats held at constant body-weight beginning at the age of three weeks there is a marked tendency to a reduction in the weight of the spleen; while at later (and longer) periods the spleen appears to undergo no material change in weight. It must be remembered, however, that the spleen is normally one of the most variable organs in the body (Jackson '13), and final conclusions should be correspondingly guarded.

In adult albino rats during chronic inanition the average loss in weight is nearly proportional to that of the entire body, while in acute inanition the loss appears very much greater (Jackson '15 a, '15 c).

STOMACH AND INTESTINES

The stomach and intestines, including mesentery and pancreas, are considered both with contents (table 19 a) and empty (table 19 b). Considering first the empty canal, it appears in rats held at constant body-weight from the age of three weeks to increase from about 4.8 per cent of the body-weight to 8.0 per cent at six and eight weeks, decreasing to 6.0 per cent at ten weeks. In absolute weight the increase would be from about 1.13 grams (1.20 grams less correction on account of difference in body-weight) at three weeks to 1.45 grams at ten weeks, an increase of about 28 per cent. Between six and thirty-two weeks there is apparently but little change; while from ten to thirty-five weeks there appears to be a decrease in the weight of the alimentary canal. The number of observations, however, is too small for final conclusion.

The most remarkable increase occurs between three and six weeks of age, from an absolute weight of 1.20 to 1.78 grams, an apparent increase of about 48 per cent in absolute weight! (The increase would be even greater if allowance were made for difference in body-weight). Thus, as in the case of the liver and

TABLE 19

The stomach and intestines; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
a. Including contents				
Normal at 3 weeks (Jackson '13)	49	18.7	1.78 (0.74–3.08)	9.3 (5.5–15.5)
Controls at 3 weeks	11	24.5	2.51 (1.96–3.23)	10.4 (7.8–15.4)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	3.10 (1.67–5.07)	13.9 (7.4–21.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	3.64 (2.58–4.71)	20.2 (4.5–26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	3.29 (2.26–6.83)	13.5 (10.3–20.2)
Normal at 6 weeks (Jackson '13)	42	50.0	8.05 (4.48–13.40)	15.9 (10.6–23.4)
Controls at 6 weeks	1	42.4	5.13	12.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	5.98 (4.71–7.24)	12.5 (10.8–14.2)
Normal at 10 weeks (Jackson '13)		75.0	9.00	12.0
Controls at 10 weeks[*]	6	134.0	11.32 (8.82–14.49)	8.3 (7.3–11.2)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	7.48 (5.90–8.81)	9.6 (8.1–10.4)
Controls at 32 and 35 weeks	6	189.5	11.60 (7.51–14.04)	7.4 (5.4–12.2)
b. Empty				
Normal at 3 weeks (Jackson '13)	16	20.0	0.90 (0.37–1.61)	4.5 (2.9–6.1)
Controls at 3 weeks	10	25.1	1.20 (0.74–1.69)	4.8 (3.1–6.7)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.78 (1.41–2.51)	8.0 (6.3–11.0)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	1.44 (1.38–1.51)	8.0 (7.8–8.3)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.45 (0.96–2.29)	6.0 (4.5–8.6)
Normal at 6 weeks (Jackson '13)		50.0	4.00	8.0
Controls at 6 weeks	1	42.4	3.00	7.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	3.22 (2.85–3.59)	6.8 (6.5–7.0)
Normal at 10 weeks (Jackson '13)		75.0	5.30	7.0
Controls at 10 weeks[*]	5	127.9	5.26	4.3 (3.9–4.9)
Body-weight constant 25 weeks (age of 10 to 25 weeks)	3	77.8	3.97 (3.48–4.35)	5.4 (4.7–5.9)
Controls at 32 and 35 weeks	6	189.5	9.16 (7.57–10.64)	4.9 (4.1–5.3)

[*] Body-weight of controls at 10 weeks too great for comparison with those held constant 10–35 weeks.

following the normal growth tendency, there appears to be in these retarded rats an early increase in the weight of the alimentary canal, reaching a maximum at about the age of six weeks, after which there is a decline in weight. It may be that the early increase in these organs is diminished later on account of the exhaustion of the stored food-supplies available elsewhere in the body at the beginning of the experiment. Individual organs and tissues differ greatly from each other in their relative susceptibility to attack and absorption at different times during the course of inanition.

The behavior of the stomach and intestines weighed with contents (table 19 a) is very similar to that of the empty canal. Contrary to what might be expected, during constant body-weight, with a restricted food-supply (water *ad libitum*), the canal does not decrease in contents. On the contrary there is an *increase* in contents (watery or mucous in character) which may even exceed in relative weight the normal contents in full-fed aminals. The maximum occurred in animals held at constant body-weight from the age of three to eight weeks, when the canal with contents formed 20.2 per cent of the body-weight! In experiments beginning at later ages and extending over longer periods (six to thirty-two weeks and ten to thirty-five weeks), the canal with contents appears to remain more nearly uniform in weight, with some tendency to decrease.

During both acute and chronic inanition in adult albino rats, there is a very marked decrease in the weight of the stomach and intestines, both with and without contents (Jackson '15 a, '15 c).

SUPRARENAL GLANDS

The suprarenal glands (table 20) from the age of about six weeks must be considered separately in the sexes, on account of a distinct sexual difference in their weight, as discovered independently by Hatai ('13) and myself (Jackson '13). In the earlier ages, however, there is no apparent sexual difference, hence the sexes are combined.

TABLE 20

The suprarenal glands; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks* (Jackson '13)	49	18.7	0.0072 (0.0040–0.0140)	0.0400 (0.023–0.074)
Controls at 3 weeks*	11	24.5	0.0088 (0.0060–0.0108)	0.0370 (0.025–0.055)
Body-weight constant 3 weeks* (age of 3 to 6 weeks)	7	22.1	0.0089 (0.0070–0.0110)	0.0400 (0.034–0.050)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1m	18.1	0.0109	0.0610
	1f	17.8	0.0088	0.0490
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7m	25.2	0.0101 (0.0072–0.0126)	0.0420 (0.033–0.052)
	12f	23.3	0.0117 (0.0090–0.0147)	0.0510 (0.040–0.061)
Normal at 6 weeks* (Jackson '13)	42	50.0	0.0128 (0.0070–0.0190)	0.0260 (0.015–0.039)
Control at 6 weeks	1f	42.4	0.0090	0.0210
Body-weight constant 26 weeks (age of 6 to 32 weeks)	1m	43.7	0.0105	0.0240
	1f	50.5	0.0090	0.0180
Normal at 10 weeks (Jackson '13)	20m	117.0	0.0220 (0.0150–0.0336)	0.0185 (0.011–0.025)
	23f	99.0	0.0253 (0.0180–0.0329)	0.0257 (0.017–0.033)
Controls at 10 weeks	3m	154.7	0.0295 (0.0270–0.0326)	0.0190 (0.017–0.023)
	3f	114.0	0.0318 (0.0290–0.0346)	0.0280 (0.027–0.031)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	2m	79.6	0.0149 (0.0147–0.0150)	0.0190 (0.017–0.020)
	1f	74.2	0.0145	0.020
Controls at 32 and 35 weeks	3m	218.7	0.0270 (0.0180–0.0310)	0.0100 (0.009–0.013)
	3f	160.3	0.0320 (0.0280–0.0360)	0.0200 (0.017–0.024)

* No sexual difference apparent.

TABLE 21

The kidneys; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.271 (0.169–0.531)	1.44 (1.19–1.87)
Controls at 3 weeks	11	24.5	0.393 (0.314–0.487)	1.62 (1.33–2.16)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.396 (0.328–0.487)	1.80 (1.46–2.05)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.339 (0.331–0.347)	1.89 (1.86–1.92)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.404 (0.325–0.515)	1.69 (1.44–1.97)
Normal at 6 weeks (Jackson '13)	42	50.0	0.616 (0.500–0.943)	1.25 (1.00–1.55)
Controls at 6 weeks	1	42.4	0.570	1.35
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.613 (0.581–0.645)	1.30 (1.28–1.33)
Normal at 10 weeks (Jackson '13)		75.0	0.750	1.00
Controls at 10 weeks*	6	134.0	1.200 (0.953–1.481)	0.91 (0.81–0.99)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.753 (0.728–0.784)	0.97 (0.92–1.02)
Controls at 32 and 35 weeks	6	189.5	1.539 (1.275–1.909)	0.81 (0.71–0.86)

* Controls too heavy for comparison.

In the rats held at constant body-weight from the age of three weeks the suprarenal glands appear to increase slightly in weight at six and eight weeks; and especially at ten weeks, where the characteristic sexual difference has distinctly appeared. In absolute weight, the suprarenal glands have increased from 0.0090 gram (0.0088 gram, plus correction for difference in body-weight; or 0.037 per cent of the body-weight) at three weeks to 0.0101 gram (0.042 per cent of the body-weight) in the male at ten weeks, an increase of about 12 per cent in absolute weight. In the female, the corresponding increase is from 0.0084 gram (0.0088 gram, less correction for difference in body-weight) (or 0.037 per cent of the body-weight) to 0.0117 gram (0.051 per cent of the body-weight), an increase of 39 per cent in absolute weight. Normally the suprarenals during this period are decreasing in relative (percentage) weight. In the experiments at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks), the changes are apparently not great, but a larger number of observations is necessary before definite conclusions can be reached.

In adult rats during inanition there is little or no loss in the absolute weight of the suprarenal glands, which therefore increase markedly in relative (percentage) weight (Jackson '15 a, '15 c).

KIDNEYS

The kidneys (table 21) in rats held at constant body-weight from the age of three weeks show a tendency to increase which is more marked at six and eight than at ten weeks. Between three and ten weeks of age the increase is from an average of 0.388 gram (0.393 gram, less correction on account of difference in body-weight; or 1.62 per cent of the body-weight) to 0.404 gram (1.69 per cent of the body-weight), an increase of only about 4.1 per cent in absolute weight. At later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there is apparently but little change in the weight of the kidneys. The slight differences shown in the table are probably not significant. On the whole, it appears that in young rats held at constant body-weight there is a slight tendency to increase in the weight of the kidneys, in the earlier weeks, but little or no apparent difference later.

In adult rats during acute and chronic inanition, the kidneys lose in weight relatively slightly less than the body as a whole, thereby gaining slightly in relative (percentage) weight (Jackson '15 c).

TESTES AND EPIDIDYMI

The weights of testes and epididymi unfortunately were not separated in some cases, which (together with their variability and the small number of observations) makes conclusions somewhat difficult. For the testis (table 22 a), the clearest case is in rats held constant from three to ten weeks of age, in which

TABLE 22

The testes and epididymi; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
a. Testes				
Normal at 3 weeks (Hatai '13)		20.0*	0.090	0.45
Normal at 3 weeks (incl. epididymi) (Jackson '13)	24	21.2	0.134† (0.076–0.224)	0.63* (0.53–0.78)
Controls at 3 weeks	6	25.0	0.144 (0.114–0.200)	0.57 (0.49–0.62)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	2	22.1	0.133† (0.129–0.137)	0.66* (0.65–0.67)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	18.1	0.176†	0.98*
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.193 (0.106–0.331)	0.74 (0.49–1.05)
Normal at 6 weeks (Hatai '13)		50.0*	0.402	0.80
Normal at 6 weeks (Jackson '13)	20	49.0	0.592† (0.368–0.958)	1.19* (0.88–1.72)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	1	43.7	0.370†	0.84*
Normal at 10 weeks (Hatai '13)		70.0*	0.774	1.11
Normal at 10 weeks (Jackson '13)	20	117.0	1.747† (0.678–2.62)	1.51* (0.63–2.41)
Controls at 10 weeks	3	154.7	1.850 (1.608–2.10)	1.21* (1.06–1.49)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	2	79.6	1.294† (1.401–1.188)	1.62* (1.61–1.64)
Controls at 32 and 35 weeks	3	218.7	1.844† (1.766–1.932)	0.84* (0.74–0.90)
b. Epididymi				
Normal at 3 weeks (Jackson '13) (estim	ated)	20.0	0.200 (?)	0.10 (?)
Controls at 3 weeks	6	25.0	0.0209 (0.0092–0.0246	0.084 (0.039–0.123)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	1	23.6	0.0144	0.061
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.0209 (0.0084–0.0360)	0.080 (0.040–0.115)
Controls at 10 weeks	3	154.7	0.4700 (0.4480–0.4920)	0.310 (0.290–0.330)

* Gross body-weight.

† Including epididymi.

there is an increase from 0.144 gram (0.57 per cent of the body-weight) to 0.193 gram (0.74 per cent of the body), an apparent increase of 34 per cent in the absolute weight. (The average net body-weight was about the same in both cases, 25.0 grams in the controls, and 25.2 grams at ten weeks).

For the epididymi (table 22 b), the conclusions are even more uncertain; but there appears to be a slight loss in the relative weight of the epididymi in rats held at constant body-weight from the age of three to six and ten weeks.

For testis and epididymis combined (table 22 a) there appears to be an increase in rats held at constant body-weight, excepting the period from six to thirty-two weeks.

While no final conclusions can be drawn, the evidence indicates that in young rats held at constant body-weight there is an increase in the weight of the testis, but not in the epididymis.

During inanition in adult rats, the testes and epididymi apparently lose weight in about the same proportion as the entire body (Jackson '15 c).

OVARIES

In rats held at constant body-weight from three weeks to ten weeks of age, there would appear to be a decrease in the weight of the ovaries (table 23) from 0.0066 gram (0.0068 gram, less correction for difference in body-weight; or 0.027 per cent of the

TABLE 23

The ovaries; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	24	16.2	0.0036 (0.0015–0.0067)	0.0220 (0.011–0.039)
Controls at 3 weeks	5	24.5	0.0068 (0.0029–0.0104)	0.0270 (0.012–0.034)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	22.4	0.0067 (0.0048–0.0084)	0.0300 (0.020–0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	12	23.3	0.0048 (0.0030–0.0064)	0.0210 (0.012–0.028)
Normal at 6 weeks (Jackson '13)	20	54.9	0.0106 (0.0050–0.028)	0.0216 (0.012–0.035)
Normal at 10 weeks (Jackson '13)	23	98.8	0.0350 (0.0100–0.070)	0.0340 (0.013–0.055)
Controls at 10 weeks	3	114.0	0.0337 (0.0297–0.038)	0.0290 (0.027–0.033)

body-weight) to 0.0048 gram (0.021 per cent of the body-weight). This would indicate a decrease of about 27 per cent in absolute weight. The ovary, however, is an organ which (like the thyroid gland) is quite variable and somewhat difficult to dissect out with accuracy, so a much larger number of observations would be required for a final conclusion.

HYPOPHYSIS

In the hypophysis (table 24) there is normally a sexual difference in weight, observable in rats above 50 grams in body-weight (Hatai '13). As in the case of the suprarenal glands, the hypophysis normally becomes relatively heavier in the female.

In the rats held at constant body-weight from the age of three weeks to six weeks, there is what appears to be a sexual difference, the one male with a hypophysis of 0.0016 gram (0.0068 per cent of the body-weight), while the two female hypophyses each weigh 0.0018 gram (0.0079 per cent of the body-weight). These are probably mere accidental variations, however, as in the three to ten weeks series the difference in the relative weight is insignificant (0.0084 per cent of the body-weight in the males, and 0.0086 per cent in the females). In any event, however,

TABLE 24

The hypophysis; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal at 3 weeks* (Hatai '13)		25.0†	0.00175	0.0070
Controls at 3 weeks*	9	25.5	0.00178 (0.0012–0.0022)	0.0070 (0.0052–0.0084)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	1m.	23.6	0.0016	0.0068
	2f.	22.9	0.0018	0.0079 (0.0076–0.0082)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7m.	25.2	0.0021 (0.0016–0.0024)	0.0084 (0.0057–0.0110)
	11f.	23.6	0.0020 (0.0010–0.0033)	0.0086 (0.0043–0.0135)
Normal at 10 weeks (Hatai '13)	m.	130.0	0.0054	0.0042
	f.	130.0	0.0084	0.0065
Controls at 10 weeks	3m.	154.7	0.0062 (0.0059–0.0067)	0.0040 (0.0038–0.0042)
	3f.	114.0	0.0054 (0.0046–0.0067)	0.0048 (0.0040–0.0062)

* No sexual difference apparent.

† Gross body-weight.

there appears to be a distinct tendency to an increase in the weight of the hypophysis in rats held at constant weight from three to ten weeks of age. In absolute weight, this corresponds in the male to an increase from 0.00178 gram to 0.0021 gram, an increase of about 18 per cent. In the female, the corresponding increase is from 0.00168 gram (0.00178 gram, less correction for difference in body-weight) to 0.0020 gram, an increase of 19 per cent.

As already shown, the suprarenal glands differ from the hypophysis in that they undergo a marked sexual differentation in weight during their growth while the body-weight is held constant. The impulse to sexual differentiation in these glands therefore appears stronger in the suprarenals than in the hypophysis. Final conclusions should be guarded, however, until a larger number of observations is available.

In the adult rat during inanition the weight of the hypophysis decreases in nearly the same proportion as the body-weight (Jackson '15 c).

DISCUSSION

With reference to their growth tendency in young rats held at constant body-weight, the organs may be divided into three classes: (1) those in which the growth tendency is so strong that they continue to increase, even when the body-weight is held constant; (2) those which approximately hold their weight constant under these conditions; and (3) those which are unable to maintain themselves and lose in weight.

According to this scheme, the organs in rats held at constant body-weight from the age of three to that of ten weeks are grouped in table 25. No grouping of this sort can be entirely satisfactory, because in some cases organs are intermediate in position, and especially because (as has been shown) in many cases the weight of an organ will vary according to the age at which the experiment was begun and the length of the period. For example, the liver, which in the three-to-ten weeks experiment shows but a slight gain, shows a larger gain at earlier times, and a loss at later and longer periods.

On the whole, however, the grouping suffices to give a good view of the results. Group I shows the organs with marked growth tendency to be the skeleton, eyeballs, spinal cord, alimentary canal, testes, hypophysis and suprarenal glands. Group II, which approximately maintains constant weight, includes the musculature, brain, heart, kidneys and liver. Group III, those which fail to maintain their weight when the body-weight is held constant, includes the integument, lungs, thyroid gland, ovaries, spleen and thymus.

In the same table 25 for convenience of comparison are also grouped the various organs of the adult rat according to their relative loss in weight during chronic inanition (Jackson '15 c).

TABLE 25

Comparison of growth tendency in young rats held at constant body-weight from age of three to ten weeks with tendency to maintenance in adult rats during chronic inanition. The figures indicate the apparent average percentage gain or loss in absolute weight during the period of experiment

	GROUP I		GROUP II		GROUP III	
	Marked tendency to growth in young held at constant body-weight. Strong tendency to maintenance in adult chronic inanition		Weight nearly constant in young held at constant body-weight. Relative loss similar to that of entire body during adult chronic inanition		Marked loss in weight in young held at constant body-weight. Relative loss greater than that of entire body during adult chronic inanition	
		per cent of change		*per cent of change*		*per cent of change*
Young at constant body weight....	*eyeballs	+50.0	liver	+10.3	lungs	−15.0
	*spinal cord	+36.0	*kidneys	+4.1	thyroid gland	−24.0
	testes	+34.0	*musculature	+3.0	ovaries	−27.0
	*skeleton	+28.0	brain	−0.5	integument	−36.0
	alimentary canal	+28.0	*heart	−0.6	spleen	−42.0
	*suprarenal glands	M. +12.0 F. +39.0			thymus	−90.0
	hypophysis	M. +18.0 F. +19.0				
Adults during chronic inanition (loss of body-weight 36 per cent)	*skeleton	+1.8	hypophysis	−25.3	liver	−43.0
	*spinal cord	−4.0	*kidneys	−26.8	alimentary canal	−57.0
	*eyeballs	−5.8	spleen	−29.0		
	brain	−6.6	*heart	−32.8		
	*suprarenal glands	−8.9	integument	−38.5		
	thyroid gland	−21.8	lungs	−40.0		
	thymus	(?)	testes	−40.3		
			*musculature	−40.8		

* Indicates correspondence between the groupings in young and adult series.

This enables us to answer the question as to whether the organs in young animals held at constant body-weight (and thus subjected to a chronic inanition) behave in a manner similar to adult rats during chronic inanition. In many cases, the grouping shows an agreement. The skeleton, eyeballs, spinal cord and suprarenal glands have a strong growth tendency in young held at constant body-weight, and also a strong tendency to maintain their original weight in adults subjected to chronic inanition. The musculature, heart and kidneys approximately maintain their weight in young held constant, and maintain their *relative* weight in adults during chronic inanition. In the majority of cases, however, the behavior of organs in the young differs materially from that in the adult. Thus the alimentary canal has a marked growth in the young at constant weight, yet it loses heavily during adult inanition. The converse is apparently true of the thyroid gland. To a greater or less degree, this inconsistency is seen in the case of most of the organs, as is evident from table 25.

This inconsistency is perhaps to be explained in the following manner. In the adult during inanition the various organs lose weight relatively in inverse ratio to the ability of their cells to extract nutrition from the diminishing quantity available in the surrounding medium and to maintain equilibrium under these adverse conditions. In the young animal held at constant body-weight, the conditions differ in that the corresponding cells have the capacity not only to *maintain* themselves, but to *grow*. The growth capacity, as is well known, is different from and to some extent independent of the maintenance capacity. The adult has lost the capacity to grow, whereas the young animal has both the powers of growth and maintenance. Hence their organs behave differently during inanition. The alimentary canal, for example, apparently has a strong growth tendency, but a weak power of maintenance.

It is further evident, however, that even the growth capacities of the various tissues and organs differ relatively from each other under different planes of nutrition. Thus under normal conditions of growth in the rat between three and ten weeks of age the *muscular* system shows the strongest growth capacity, and increases with greater relative rapidity than any other system (Jackson and Lowrey). The skeleton is of course growing steadily, but at a much slower rate, so that it is decreasing in relative (percentage) weight. Under the adverse nutritive conditions in young animals when the body-weight is held constant, however, the musculature is barely able to maintain its weight, while the skeleton is able to absorb more than its share of the available nutrition, and to grow steadily (though at a retarded rate). There are similar differences among many of the individual organs, though in some cases (e.g., liver, alimentary canal) there is a certain degree of parallelism between the normal growth tendency and the behavior when the body-weight is held constant at corresponding periods.

That also the power of maintenance may vary in organs according to the nutritional conditions is shown by the characteristic differences in the losses of organ-weight in chronic inanition as compared with acute inanition in adults (Jackson '15 a, '15 c).

Finally, it should be remembered that the results of the present paper, as well as those concerning adult chronic inanition in a previous paper (Jackson '15 c), are based upon the use of a diet wholesome and balanced, but insufficient in quantity. It is probable that more or less different results would follow from other forms of inanition, such as 'partial inanition' from a chemically defective or highly unbalanced diet. For example, Hatai ('15) finds a pronounced atrophy of the testis and other characteristic changes in albino rats whose growth had been retarded by a 'lipoid-free' ration. Bowin ('80), however, in dogs and rabbits fed dry food only (no water) found, with a loss of about 50 per cent in body-weight, the losses in organ-weight similar to those following total hunger.

SUMMARY

The principal results of the present paper may be summarized briefly as follows:

Young albino rats may be held at constant body-weight for considerable periods by underfeeding. The amount of food required for this purpose decreases as the experiment proceeds.

As to the body-proportions, the relative weights of the head, trunk and extremities remain practically unchanged during the experiment. There is apparently a slight increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to seem of doubtful significance.

Of the systems—integument, skeleton, musculature, viscera and 'remainder'—there is but little change in the weights of the musculature, visceral group (as a whole) and 'remainder.' There is, however, a marked decrease in the weight of the integument, counterbalanced by a marked increase in the skeleton. Thus on the low plane of nutrition in the young body maintained at constant weight, the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process of corresponding ages, during which the musculature increases with relatively great rapidity and the skeleton lags behind relatively.

The increase in the skeleton during constant body-weight appears to involve the ligaments as well as the cartilages and bones. The skeletal growth tends to proceed along the lines of normal development, as indicated by decrease in the water-content, and by formation and union of various epiphyses. Another evidence of the tendency to normal development of the skeleton is seen in the increased relative length of the tail as compared with the body-length. The teeth also continue to develop normally (formation and eruption of the third molars).

The individual viscera may be classified in three groups:

(1) There is during the maintenance of constant body-weight in young rats a well-marked increase in the weights of the eye-

balls, spinal cord, alimentary canal (both empty and including contents), testes, hypophysis and suprarenal glands. The suprarenals undergo sexual differentiation in weight (as occurs normally), but the hypophysis apparently does not.

(2) There is no marked change in the weights of the brain, heart, kidneys and epididymi. The liver is variable, showing a definite increase in the earlier periods, but a decrease later. The lungs show a slight decrease in the early periods, but not in later.

(3) There is a well-marked decrease in the weights of the thymus ('hunger involution'), spleen, thyroid gland and ovaries.

When the organs are similarly grouped according to degree of loss during chronic inanition in the adult (slight loss, loss proportional to body, and loss greater than body), many differences are found on comparison with the corresponding groups in the young during constant body-weight. This is explained as due to to the presence of both the growth tendency and the (more or less different) maintenance tendency in the young animals, whereas in the adult there is only the tendency to maintenance. Both the growth tendency and the maintenance tendency, however, show characteristic differences in the various organs according to nutritional conditions (normal nutrition, acute or chronic inanition).

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OBSTACLES TO RESEARCH[1]

THE duty of the university to investigate the unknown as well as to teach the known is clearly evident. In the performance of this duty, the importance of research work is emphasized in many ways. Promise of productive scholarship is a leading qualification demanded in selecting members of the faculty. Encouragement and facilities for original work are freely provided. And yet we must confess that the outcome, broadly speaking, is somewhat disappointing, both here and in other universities. It is true that the results in some departments and in many individual cases are satisfactory. On the whole, nevertheless, considering our great opportunities, we seem to add relatively little to the sum total of human knowledge. Why? A recognition of the obstacles to research might enable us in some measure to overcome them. At any rate, the problem is worthy of our earnest and careful consideration.

Let us consider the problem from the biological point of view. The accomplishment of every human being (as of all living things) is the resultant of two factors: heredity and environment. In research work, as in all other lines of activity, the *limits* of possible achievement for each individual depend upon his innate talent, established through heredity. Within these limits, however, the *realization* of possibilities is conditioned by the environment. We must therefore distinguish clearly between (possible) capability or capacity and (actual) accomplishment in the field of original investigation.

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[1] An address delivered before the Minnesota Chapter of the Sigma Xi Society, October 21, 1915.

The first and most important obstacle in research work is accordingly the limitation of capacity, which is determined by heredity. Since it is now too late to quarrel with our ancestors concerning the matter, as individuals we may as well recognize this as an insurmountable obstacle. From the broad university point of view, this fundamental obstacle may be partially removed by great care in the selection of faculty members. Geniuses are scarce, however, and competition for them very strong; so it is inevitable that even in the strongest universities the faculties must be made up of men with varying degrees of innate talent.

But while our heredity is beyond our control, our environment is not. At least we can modify the environment to a considerable extent. And this is a fact of tremendous practical importance. After all, environment does play an important part in determining both the quantity and the quality of our performance in all lines, including research work. If the environment is sufficiently unfavorable, even the highest genius is sterile. Of two men with equal native ability, one with better opportunity may be far more richly productive than the other. It is a case of seed and soil. The result is determined by heredity plus environment; or perhaps better, heredity *times* environment.

Geniuses are sometimes able to accomplish a great deal, even in a relatively unfavorable environment; but fortunately research work is not a province reserved exclusively for genius. It is encouraging to most of us to remember that the army of investigation requires private soldiers, as well as officers of various grades. Even moderate capacity does not preclude research work of real value. As expressed by John Hunter:

> A man with a sufficient fund of knowledge, and a close application to one art or science, will make

great improvements in it though his talents may not be the best; or, in other words, though he be not a great genius.

Conquering the unknown in the field of knowledge is somewhat like civilization invading a new territory. A few bold and talented explorers may lead the way and blaze out new paths in the wilderness; but their excursions would be fruitless unless followed up by pioneer settlers, who by arduous labor develop the country and render its resources available for mankind. Moreover, even the explorer is in many ways largely dependent upon the knowledge and equipment furnished by others, his predecessors and his supporters.

Likewise, in the exploration of the field of knowledge, there is work for all. The history of science abundantly proves that brilliant discoveries and important generalizations usually rest upon a long series of accurate observations, requiring care and patience, but not great genius. A classical example is that of Kepler's laws of planetary motion, founded upon the extensive astronomical observations by Tycho Brahe. In biology, to substantiate and support the cell-doctrine of Schleiden and Schwann, and the doctrine of organic evolution of Darwin, has required an immense amount of patient labor by a multitude of observers during the past century. Other examples could easily be cited in various fields. The plodders as well as the geniuses should receive their due share of credit for the progress of science.

The production of research work of merit is thus within the capabilities of every one worthy of membership in a university faculty. Doubtless some who are talented predominantly as teachers should devote themselves chiefly to this field, and others are especially fitted for administrative work; but it is desirable that every one should participate to at least a slight extent in research work. As a matter of fact, we may go still further in urging that the *spirit* of scientific research should pervade *all* education, from the kindergarten to the university. Mankind in general is still far from appreciating the fact that the method of science is not a mysterious gift of genius, but a practical tool in the discovery of facts and in their application to the problems of everyday life. As Professor Remsen so aptly expressed it in his address at the dedication of the chemistry building of the University of Minnesota last year, the scientific method is essentially this: "First study the facts; then draw your conclusions from them."

From this point of view, all our problems thus become research problems; and education is able to teach us how to solve them efficiently in proportion to the extent to which training is provided in the methods of original investigation. Thus all education should provide training in scientific research, differing in degree rather than in principle as we pass from elementary to higher education. President Hill (in a recent commencement address at the University of Minnesota) has well said that "The teacher should arouse the spirit of discovery as the first step in the process of learning." A more general recognition of the significance of scientific research for education, a correction of the prevalent error that research is a matter concerning only a chosen few, would remove an obstacle which prevents a more generous support of higher scientific investigation.

While all instruction should be permeated with the research spirit, a conscious effort should be made, especially in the university, to single out as early as possible those students showing unusual talent for original work, and to give them particular aid and encouragement. We must constantly emphasize the necessity for recognition of unusual talent, since otherwise our entire time and energy will tend to be exhausted in caring for the larger number representing mediocrity. This subject is well discussed in a recent report of the subcommittee on the selection and training of students for research (Committee of One Hundred of the American Association for the Advancement of Science), published in SCIENCE, September 17, 1915.

It is, however, not my present purpose to consider the message of science for education in general, but rather to discuss the specific obstacles met by university workers in the field of original investigation. Since we can not change our heredity, possibilities for improvement must be found in the environment. What factors in our environment affect our scientific productivity?

We might classify the environmental factors affecting our research work in two groups: mental and physical. In the first rank, I would place the factors determining our mental attitude toward research. I suspect that investigation lags more frequently from lack of sustained interest than from any other cause. It is doubtless true that one is usually most interested in what one can do especially well. And research ability, as we have already noted, is largely a hereditary matter. Nevertheless, our mental attitude is unquestionably influenced in large measure by the opinion of our colleagues. Appreciation by one's fellows is a most powerful stimulus. Thus a general recognition of good research work will greatly encourage the worker to persist in spite of all obstacles. If Sigma Xi can succeed in establishing a more enthusiastic *esprit de corps* among investigators, it will greatly help the cause of scientific research.

The physical factors affecting research work are also of importance. The obstacles under this group include lack of material facilities, lack of time and lack of organization. Each of these may be briefly considered in turn.

The material facilities necessary for research include laboratories and equipment of various kinds, supplies, instruments, technical assistants, books, etc. It is customary to cite lack of adequate facilities of this kind to explain shortcomings in scientific productivity. And there is no doubt that more generous provision for these things would greatly facilitate many lines of research work. But, generally speaking, I believe that this factor is somewhat overrated. The man who does nothing because facilities are inadequate would usually accomplish but little even with unlimited resources. On the other hand, the man whose heart is in his research work will rarely fail to secure adequate support, if he perseveres and demonstrates his interest and capability.

Inadequate support of research work is sometimes ascribed to lack of appreciation on the part of university administrative officers, who control the purse-strings. This is usually an unjust accusation. University officers as a rule are keenly anxious to encourage and support research work, but they in turn are always more or less hampered by financial limitations. With the present evidently increasing popular interest in and appreciation of scientific work, however, we may confidently expect in the future more generous provision of funds available for this purpose. Even the "man in the street" can see how Germany has increased her efficiency by systematic encouragement of scientific research. America is likewise beginning to realize that this is not a luxury but a necessity, for which generous support must be provided.

Even more than lack of facilities, lack of time is an obstacle very frequently encountered by university research workers. Many university men are carrying a burden of routine teaching which, if well done, must greatly encroach upon the time absolutely essential for serious research work. In many cases, a considerable amount of routine administrative duties, committee work, etc., is added. Under these conditions, which shall be neglected—teaching, administrative work, or research? Or should one risk the danger of overwork by trying to keep up with all? Surely this is a question hard to answer. The proper solution is of course to provide a sufficient staff to handle the routine teaching and administration, and at the same time leave adequate time free for research. In a rapidly growing university, however, it is difficult to make this provision. But conditions are improving in this respect, and comparatively few men are so overburdened with routine work as to preclude a reasonable amount of time for research.

Lack of time for research work is often due not so much to the actual amount of other work as to waste of time. By carefully planning our university work, much time could be saved. There is too much "scatterment." All too frequently we allow minor routine duties to break in at all times. These minor details should be concentrated so far as possible at certain designated periods, so as to leave uninterrupted consecutive time free for research. A set of office-hours established and rigidly kept will gain a surprisingly large amount of time otherwise frittered away. Thus one serious obstacle to research may be readily removed.

Finally, I believe that another obstacle of importance in many cases is the lack of a proper organization of the research work itself. For the best results, careful, systematic planning is necessary. Too often investigation is taken up in a haphazard sort of way, which is likely to result in failure. While no rule can be made which will apply to all cases, it is certainly true that the topic to be investigated should be carefully considered before the work is undertaken. The literature should be scanned sufficiently to make sure that the contemplated problem has not already been solved, and to render available the experience of others in similar fields. Work should not be undertaken until the necessary facilities are assured to carry it through. In general, a broad fundamental problem of which successive phases may be worked out through a series of years will prove more profitable than a number of shorter, unrelated subjects of investigation. Wherever possible, cooperation with one's students or colleagues in research will usually yield better results, from the standpoint of economy in time and cost, than will individual efforts. Such matters may seem self-evident to some and trivial to others; but I feel sure that in many cases more attention to them would be well worth while. In short, system is as necessary for efficiency in research as in any other kind of work.

In conclusion, the main points may be emphasized as follows: Obstacles to achievement in research are due partly to inherent or hereditary limits of capacity, and partly to environmental factors. The latter, which are to some extent within our control, include factors determining the mental attitude, which is of primary importance. The remaining factors include the material facilities, increased support for which depends chiefly upon better appreciation by the public of the value of scientific work. Lack of time is often another important obstacle, which in part may be overcome by a more economic arrangement of routine duties. Finally an obstacle in many cases is the lack in the research work itself of systematic planning and organization, which is necessary for the highest efficiency.

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THE NERVUS TERMINALIS IN MAN AND MAMMALS[1]

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NINE FIGURES

It is over nineteen years since Pinkus ('94) first called attention to a 'new nerve' attached to the telencephalon of Protopterus, and thirty-five years since the first record of this nerve having been seen in a shark (Fritsch '78). The forms in which this nerve has now been recorded and its chief characters have been briefly summarized in the writer's previous communication ('13). In that paper the existence of a true nervus terminalis in human and certain mammalian embryos was clearly established. At the same time McCotter ('13) pointed out the existence of the nerve in the adult cat and dog. Huber and Guild ('13) have since studied the peripheral relations of the nerve in the rabbit in late foetal stages and during the first six days after birth.

The purpose of the present note is to call attention to the presence of the nervus terminalis in certain other adult mammals in the hope that a larger number of workers may undertake the study of its central and peripheral relations. At the present time it is clear that at least a part of the nerve is distributed to the mucosa of the nasal sac and in mammals accompanies the vomero-nasal nerve. A part of the nerve, however, in mammals clearly goes beyond the limits of the vomero-nasal organ. In the rabbit it spreads over a rather wide area of the nasal septum (Huber and Guild). The nerve is usually accompanied by ganglion cells, which Brookover ('10) believed to be sympathetic in character. Huber and Guild incline to the same conclusion. Although the central relations of the nerve have been studied by special methods by Herrick ('09), Sheldon ('09) and McKibben

[1] Neurological Studies, University of Minnesota, no. 19, November 15, 1913.

('11) it is still not known whether its fibers are all afferent, or whether some or all of the fibers arise from cells within the brain. In the latter case they might be considered preganglionic fibers of the sympathetic system. The present state of our knowledge suggests the probable presence of both afferent and efferent components in the series of vertebrates. The wide-spread presence of the nerve in adult mammals, including man, should add interest to the study of its relations.

The pig. In my previous communication it was stated that the nerve had not been seen in 73 and 90 mm. pigs. Since then it has been found by dissection in numerous pig foetuses ranging from 50 mm. to full term. The brain of the adult pig has not been examined.

The horse. Figure 1 shows the proximal portion of the right nervus terminalis in the brain of an adult. The figure shows a small portion of the basal surface of the brain between the olfactory trigon and the median fissure. A part of the anterior cerebral artery is seen in the right hand part of the figure. The plexus of small vessels lies immediately upon the brain substance, the nervus terminalis lies outside of them and is in turn covered by the pia. The nerve has about fourteen rootlets which enter the brain along the rostral and medial border of the medial olfactory tract. The rootlets unite by twos and threes and eventually form a common nerve trunk. Upon the largest one of three main roots into which the rootlets unite, as seen in the figure, there is an obvious ganglion. The nerve trunk extends forward nearly parallel with the olfactory peduncle to a point opposite the rostral border of the olfactory bulb, where it is lodged in the pial septum between the hemispheres. Here the nerve had been cut off in removing the brain from the skull.

The nerve of this side was removed after drawing and cut into three pieces for staining. The distal piece was treated with vom Rath's picro-osmo-palatino-acetic mixture, cleared in cedar oil and mounted in damar. It contains three fairly well medullated fibers and seven or eight fibers which were lightly and irregularly blackened. The middle piece was stained in a mixture of nigrosin and acid fuchsin but a differential staining of nerve fibers and connective tissue was not obtained. The proximal piece included a part but not all of the ganglion seen in figure 1. The piece was stained in neutral red. A number of cells were found scattered along this piece and the portion of the ganglion consisted of about twenty closely packed cells varying in size. All the cells had large nuclei with prominent nucleoli. Although the Nissl bodies were not clearly stained, owing to unsatisfactory fixation, there is no doubt that the cells are nerve cells. Two nerve cells were seen also in the piece stained by nigrosin.

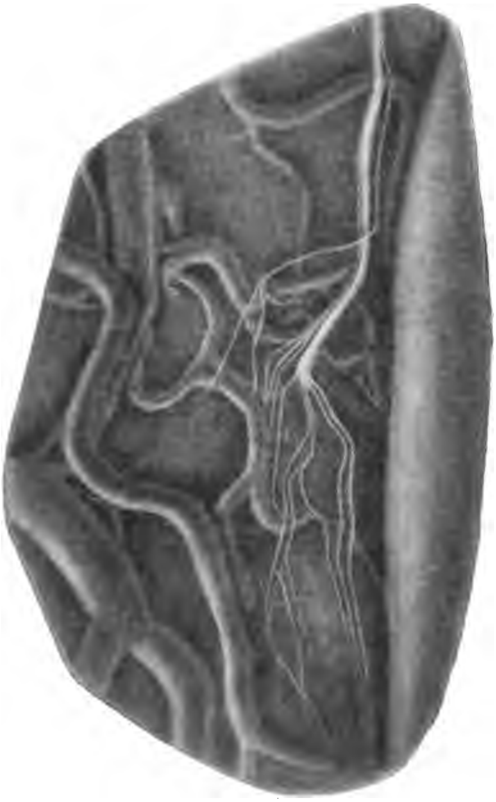
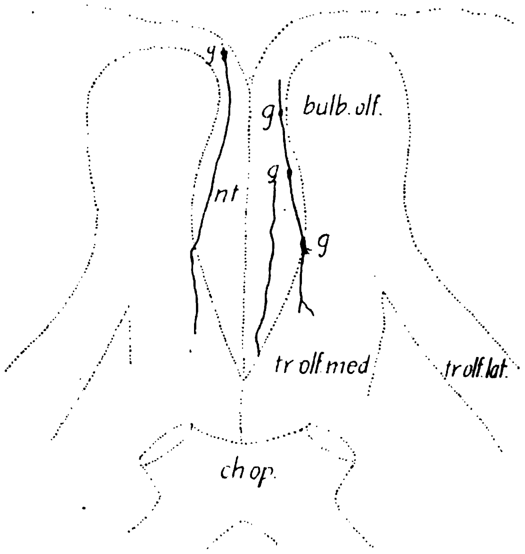


Fig. 1 Root of the nervus terminalis in the horse, right side. Description in text.

The nerve on the left side of this brain is similar to this although it differs in the number and arrangement of rootlets.

The sheep. Three brains of the adult sheep have been examined. The nerve was not found in the first but was present in the other two. In one of these brains (fig. 2) there was a single strand on the right side and two strands on the left. One of the latter strands presented three conspicuous ganglion-like enlargements. Upon staining and mounting these proved to be true ganglia. The nerve of the right side contained single ganglion cells scattered along its course, two collections of six or eight cells each and a ganglion at its distal end larger than any one of the three on the left side. A piece of the left nerve, treated in vom Rath's fluid, showed a single lightly medullated fiber.

The porpoise. I am indebted to Mr. W. F. Allen of this laboratory for the brain of a porpoise (Phocaena) preserved in Bouin's fluid. The brain of the porpoise has a very broad, rounded frontal lobe (fig. 3), the optic tracts diverge very widely and the anterior perforated space is greatly elongated from side to side. In the absence of the olfactory bulb and peduncle the topographical relations in this part of the brain must be based chiefly on the extent of the anterior perforated space. Upon the basal aspect of the frontal lobe there are seen beneath the pia seven slender strands which converge forward to a point corresponding as nearly as may be judged to the point at which the nervus terminalis was cut off in the horse's brain. Here likewise the nerve had been cut in removing the brain. Proximally the strands enter the brain over a wide area. The most lateral one enters the lateral part of the anterior perforated space. The most medial strand runs along the median fissure and bends up on the medial surface to penetrate the brain in the fissura prima on this medial surface (fig. 4). Two strands follow the anterior cerebral artery in the median fissure and bend laterad with it almost in contact with the rostral surface of the optic chiasma and enter the brain in the depth of the fissura prima on the basal aspect. The nerves of the left side have been described and drawn; those of the right side have a similar arrangement. The nerves are relatively larger than in the horse and are more closely applied to the brain surface throughout their course. They differ also in that the rootlets are flattened strands which run for a longer distance before uniting.



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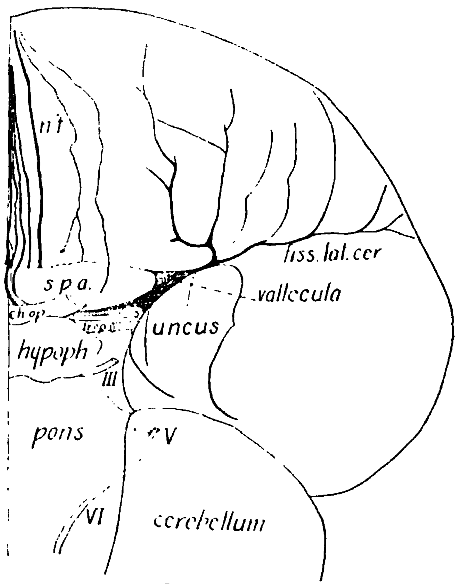

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Fig. 2 Nervus terminalis in the sheep; *g*, ganglia; *ch.op.*, optic chiasma.

Fig. 3 Basal aspect of the brain of the porpoise; *s.p.a.*, substantia perforata anterior. The broken line bounding this rostrally marks a small sulcus occupied by a blood vessel. Three of the rootlets penetrate the brain beneath this vessel. *X* indicates point at which a root was broken in dissection.

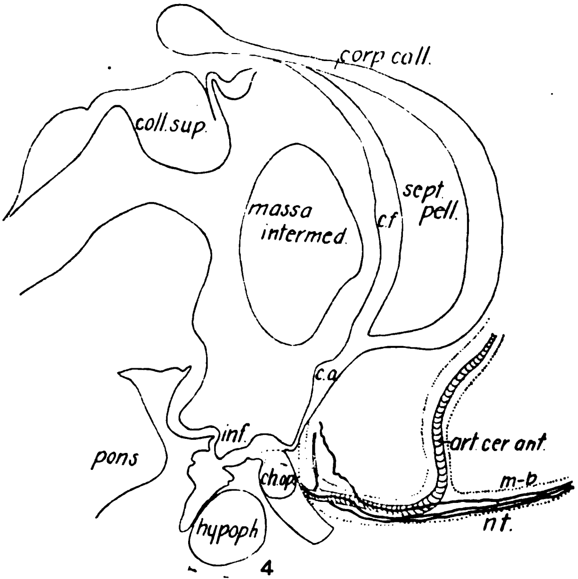
The monkey. The brain of Macacus rhesus and that of M. cynomolgus have been examined. Both show the nervus terminalis in characteristic form (fig. 5). Rostrally several nerve strands converge on the orbital surface of the frontal lobe (gyrus rectus) between the olfactory bulb and the median fissure and are cut off opposite the anterior end of the bulb. Traced proximally these strands diverge over the surface of the gyrus rectus and bend down into the fissura prima. The number of rootlets is greater in the rhesus, but otherwise the arrangement is essentially the same. The most mesial and thickest strand was removed from the rhesus brain, treated with vom Rath's fluid and stained with carmalum. The strand proved to be so compact that it could not be teased out with needles and only its proximal and distal portions could be examined satisfactorily. The rootlets contained no medullated fibers but in the distal one-fourth of the nerve medullated fibers began to appear and increased until there were fourteen to be seen at the distal end of the portion mounted. Near the distal end was seen a single large, typical ganglion cell. Several small cells in both the proximal and distal ends presented the appearance of nerve cells.

Man. I have examined a foetus of the fifth month, one of seven months, one at full term, a baby of four months and fourteen adult brains.

The five-months foetus had been long in Zenker's fluid or other bichromate solution and was very brittle when it came into my hands. On removing the right hemisphere the nerve of the left side was readily seen as a good-sized whitish strand extending from the fissura prima forward parallel with the olfactory peduncle toward the septum medial to the bulb. The condition of the material made it impossible to follow the nerve peripherally.

Fig. 4 Medial aspect of part of the left half of the brain of the porpoise. The dotted line *m–b* is the line of meeting of the medial and basal surfaces of the frontal lobe. The dotted line below is the profile of the rounded basal surface of the frontal lobe. The three more medial roots are shown. The most medial one enters the brain below the anterior commissure (*c.a.*); *c.f.*, fornix.

Fig. 5 Nervus terminalis in the monkey. The nerves of the right side of Macacus rhesus and those of the left side of M. cynomolgus are drawn; *f.p.*, fissura prima.


corp coll.
coll. sup.
massa intermed.
c.f.
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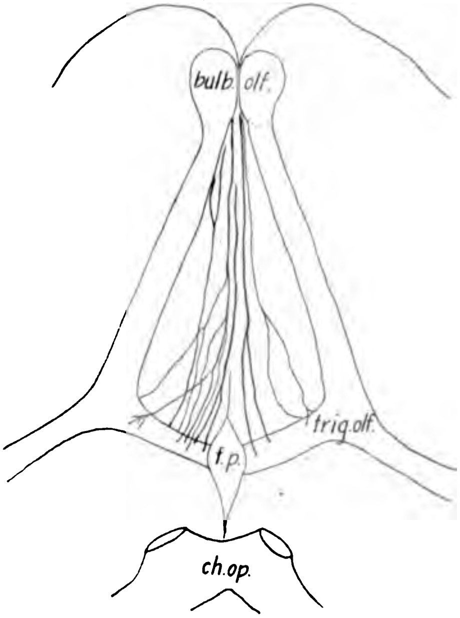

bulb. olf.
trig. olf.
f.p.
ch.op.

5

The seven-months foetus had been in formalin for at least six years and had not been fresh enough for histological study when preserved. The brain was rather soft and the tissues tough, so that the attempt to trace the nerve into the nose had to be given up. The brain was carefully removed and upon examination under the Greenough binocular, two transparent nerve strands were seen (fig. 6) upon the orbital surface of the gyrus rectus which were cut off opposite the anterior end of the olfactory bulb as in the forms above described. The apparent change in the position of the nerve since the five month stage is due to the rapid development of the frontal lobe, which has expanded mesad producing a gyrus rectus medial to the olfactory peduncle. The pre-chiasmatic space is filled with a gossamer-like fibrous tissue which had to be removed patiently in order to follow the strands to their point of entering the brain. On the left side one of the strands pierced the rostral border of the medial olfactory tract near the trigon. The other strand divided into two rootlets which ran deeper into the fissura prima (fig. 6). On the right side the nerves were not fully dissected, the anterior cerebral artery being left in position to show the relations.

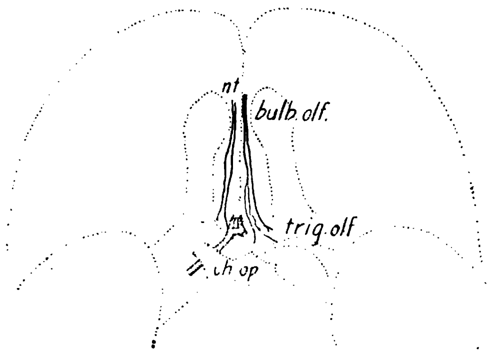


Fig. 6 Seven months human foetus, basal surface of the frontal lobes showing the nervus terminalis.

In the full-term foetus the attempt was made to trace the peripheral course of the nerve. The dissection was made from the face in order to expose the orbital surface of the frontal lobe. The nerve of the left side was found on the gyrus rectus as in other cases. It was a single thick strand readily visible to the naked eye. Traced forward it entered the median fissure opposite the anterior end of the olfactory bulb. Curving somewhat dorsad in the fissure the nerve makes a gentle curve ventrad again and at the same time leaves the surface of the brain, with which it is in contact, and enters the pia mater. At the same time the nerve divides into several strands which flatten out like a fan. These thin flat strands pierce the pia and enter the tissue of the cribriform plate close to the median plane among the most anterior strands of the olfactory nerve. Here the connective tissue was so tough, owing to the formalin preservation, that the thin strands could not be followed far. Some of them were followed without doubt into the septum, and some appeared to go toward the lateral wall of the nasal chamber, but this was uncertain. The point at which these strands pierce the pia mater is the place where the nerve is cut off when a brain is removed from the skull. The fact that at this point the nerves in the adult brains dissected were either within or very near to the median fissure and imbedded in the pia, suggested the possibility that the nerves might be distributed to the meninges, but the dissection of this specimen was carried far enough to show conclusively that they go down into the septum, and to explain their position in the adult brain.

The brain of a baby of four months showed two strands on the left and three on the right. On both sides the roots entered the brain beneath the medial and rostral border of the medial olfactory tract. The nerve of the left side was stained and mounted, but no ganglion cells were found.

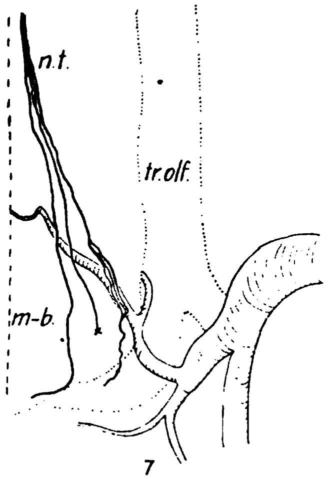
Fourteen adult human brains have been examined and the nervus terminalis found in all. In most cases the nerve is visible under the lowest power of the Greenough binocular without any dissection. It is only necessary that the pia mater shall be intact in the region between the olfactory peduncles and rostral to the optic chiasma. In only one of the fourteen brains was the nerve so small as to be at all difficult to follow. The nerve strands lie just beneath the pia and are visible through it because slightly more whitish opaque than the pia. They are distinguished from small blood vessels because the vessels, even when apparently empty, have a slightly yellowish color. Moreover, small blood vessels are readily traced to the larger ones from which they arise. It is more difficult to distinguish the nervus terminalis from thin bands of connective tissue which lie in or beneath the pia. Most of the connective tissue strands in this region are inserted in the thick tissue surrounding the anterior cerebral artery or some of its branches in the median fissure, from which they stretch obliquely forward and laterad. The connective tissue strands can be detected by pulling this way and that upon the pia with fine forceps. The strands of connective tissue will be distorted and the appearance of strands will be produced parallel with the direction of tension, while the nerve strands, if present in the area pulled upon, will not be distorted, nor obscured. When the pia mater is pricked through, lifted up with forceps and dissected away it is found that the nerve strands lie beneath the pia but are attached to it more or less closely. The attachment is by means of slender connective tissue strands and in some instances by small bands which pass beneath the nerve in the form of loops or straps. In some cases the length of the nerve was drawn through one of these loops in order to free it from the pia. With a little care the nerve is separated from the pia and a few minutes suffice to expose the nerve through the greater part of its intracranial course. The dissection of the roots where they bend around the heel-like convexity of the gyrus rectus just in front of the optic chiasma requires more care, especially when the nerve divides into several slender rootlets.

Figures 7, 8 and 9 show the course of the nerve in three adult brains. It runs over the orbital surface of the gyrus rectus and

Fig. 7 Part of the basal aspect of the left frontal lobe in the adult human brain. The blood vessel is a small vein greatly distended with blood. *X* marks a root broken in dissecting.

Fig. 8 Basal aspect of the right frontal lobe in the adult human brain. Anastomosis of rootlets.

Fig. 9 Similar to figure 8. Nervus terminalis, a single strand with five rootlets on the surface of the medial olfactory tract.



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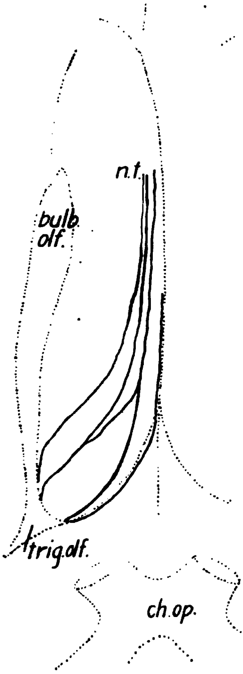

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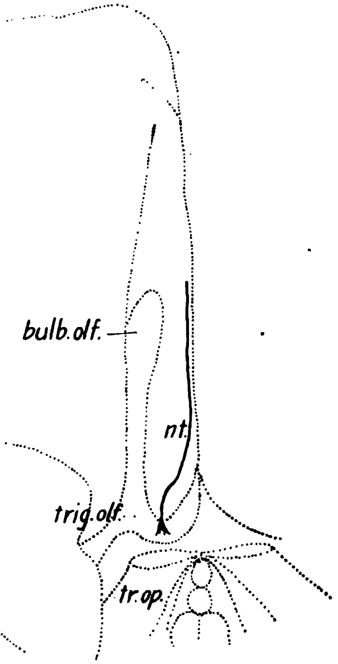

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enters the median fissure or lies near it at the level of the anterior end of the olfactory bulb, where the nerve is cut off in removing the brain. As seen in the figures, the nerve in these cases consisted of one, two, three or four strands. In one case (fig. 7) one of the strands divided into three strands which again united into one. In another case (fig. 8) a slender strand crossed obliquely from one of the main strands to another. It is entirely possible that slender strands would be overlooked or destroyed in dissecting and two or three minute strands were seen which are not included in the drawings. In the fourteen brains the type of nerve most frequently met with is that shown in figure 8. In one case the subdivision and reuniting of strands seen in figure 7 was present, together with a large number of very slender strands which ran along the medial border of the olfactory peduncle and bulb. When the nerve consists of a single strand, as in figure 9, it is large enough to be seen and dissected without the use of a lens. In one case it appeared larger than some of the rootlets of the IX and X nerves; but, being a broad thin band, it probably contains fewer fibers than those rootlets.

The point of attachment of the roots to the brain varies considerably. In all cases thus far examined it is in the region of the basal end of the fissura prima, either upon or in front of or behind the medial olfactory tract. In figure 8 the roots are seen converging toward the olfactory trigon, where they seemed to dip beneath the anterior and medial border of the olfactory tract. In figure 9 the single root divides into five rootlets which pierce the medial olfactory tract. The two roots of the other side of this brain had the same position. Two of the three roots in figure 7 have nearly the same position but one of them passes farther toward the median plane.

In position these strands correspond very closely to the nervus terminalis of lower animals and of human embryos previously described. Proof of the nervous character of the strands was sought, however, by removing and staining some of them. In one brain the nerve was found on only the right side. Its two rootlets entered the brain just at the rostral border of the medial olfactory tract where that bends from the orbital to the medial surface. The roots were pulled out and the entire nerve was stained in neutral red and mounted in damar. It consists of some fifteen or sixteen small bundles of non-medullated fibers and has imbedded in its course about twelve ganglion cells. These cells occur singly or in twos. There is no larger collection. Each cell has a large nucleus and prominent nucleolus and stains deeply with neutral red. Unfortunately the material was not fresh enough to give a good stain of the Nissl substance.

The nerve on the right side of the brain from which figure 9 was taken was removed and stained as was also the left nerve of the four months baby brain, but no nerve cells were found in either. From the brain shown in figure 8 the nerve of the left side was stained in neutral red, while that of the right side was treated with vom Rath's fluid and afterward stained with carmalum. Neither medullated fibers nor nerve cells were found in this case. The largest nerve found was treated in the same way and teased out carefully. After clearing and mounting in damar there were found four ganglion cells surrounded by numerous sheath-cell nuclei. Two cells were in the middle part of the nerve, two at the distal extremity. No medullated fibers were found. Failure to find ganglion cells in some of the other cases may have been due to the fact that the nerves were not as well teased out.

From the above facts it appears that a nerve corresponding to the nervus terminalis of lower vertebrates exists in adult man and several other mammals. This nerve contains some medullated fibers at least in the sheep, horse and monkey, and in the monkey these fibers increase in number distally. In the sheep, horse, monkey and man the nerve contains ganglion cells in at least some individuals. In the sheep and horse there are distinct ganglionic enlargements of the nerve. The failure to find ganglion cells in the other cases here reported has little significance, since only the intra-pial course of the nerve could be examined. The probability that ganglion cells would be situated farther distally is suggested by the condition in the rabbit (Huber and Guild) and by the presence of a large ganglion in the sheep (fig. 2) just at the point where the nerve pierces the pia, rostral to the olfactory bulb. The presence and large size of the nervus terminalis in the porpoise serves to emphasize its independence of the olfactory nerve, which has been abundantly established by previous work.

The nerve has usually been regarded as a vestigeal structure, but it is an interesting fact that it is larger in man than in many fishes and amphibians. The question suggests itself, for how many million years has the nerve persisted in a vestigeal condition? Further studies are desirable upon the central relations of the nervus terminalis and upon its structure and distribution in the nasal septum.

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June, 1915

A TRACTUS OLFACTO-TEGMENTALIS IN THE HUMAN FETAL BRAIN[1]

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University of Minnesota

NINE FIGURES

It is commonly taught that the most direct pathways over which impulses aroused in the olfactory centers may reach the motor centers are the two following:

a. From the olfactory centers in the forebrain to the tuber cinereum or the corpus mammillare, and from these to the tegmentum or possibly to motor centers directly;

b. From the forebrain centers to the nucleus habenulae, thence over the bundle of Meynert to the interpeduncular nucleus and thence to motor centers over paths not well known.

The following description adds to the evidence that a connection exists between the olfactory and lower centers without interruption in the diencephalon.

The brain studied is that of a fetus 145 mm. in length, crown-rump measurement. It was received fresh and fixed in 15 per cent formalin. Sections were cut transverse to the long axis of the hemispheres and lengthwise of the brain stem, 50 microns in thickness, and stained with Delafield's hematoxylin. The preservation proved to be excellent.

The brain at this age is largely composed of cell-masses which are deeply stained. The fibers take little stain and where definite bundles are present, these stand out sharply among the cell-masses and neuropile. Certain well known tracts, such as the bundles entering into the anterior commissure and the fornix, are very clearly defined in this brain.

In a section passing just rostral to the anterior commissure (fig. 4) we see on the right side the precommissural fibers of the

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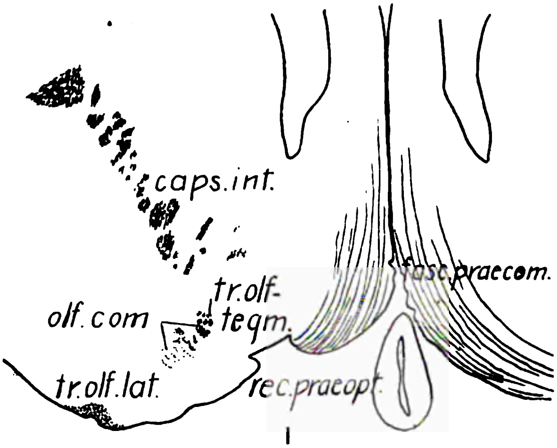
[1] Neurological Studies, University of Minnesota, No. 20, April 1, 1915.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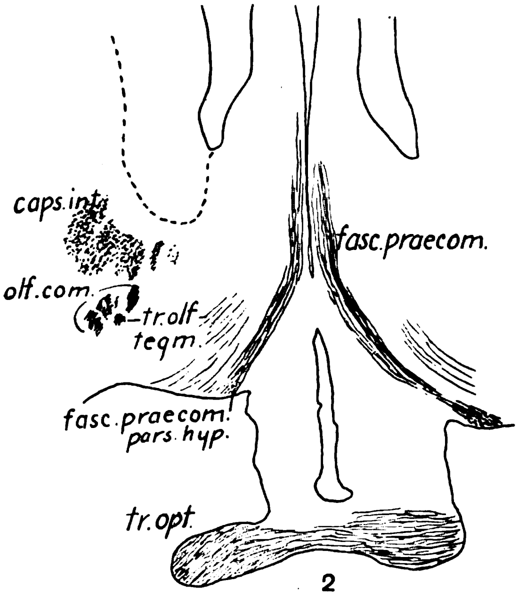
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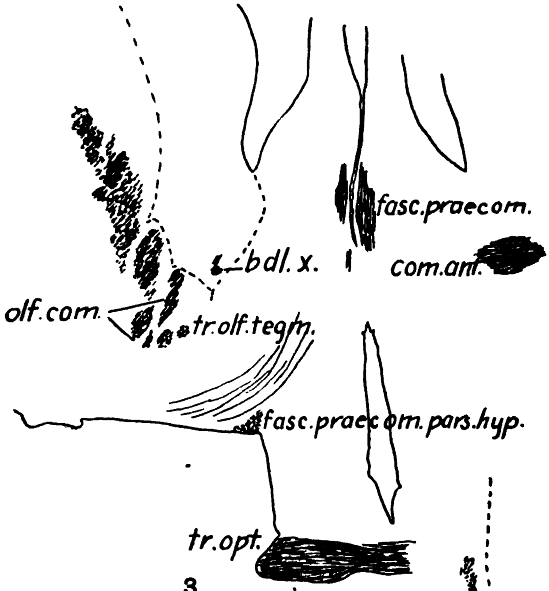
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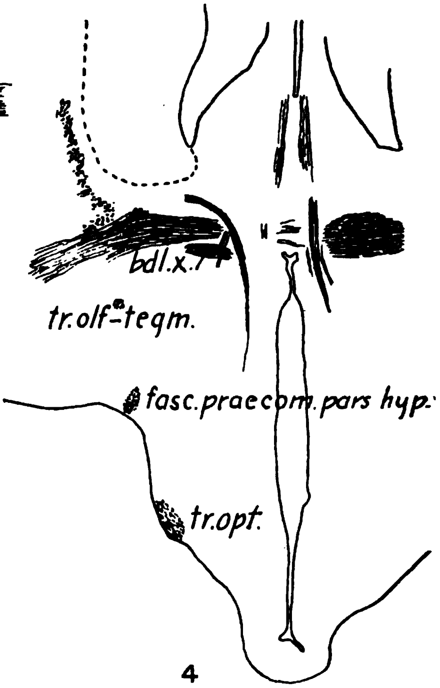
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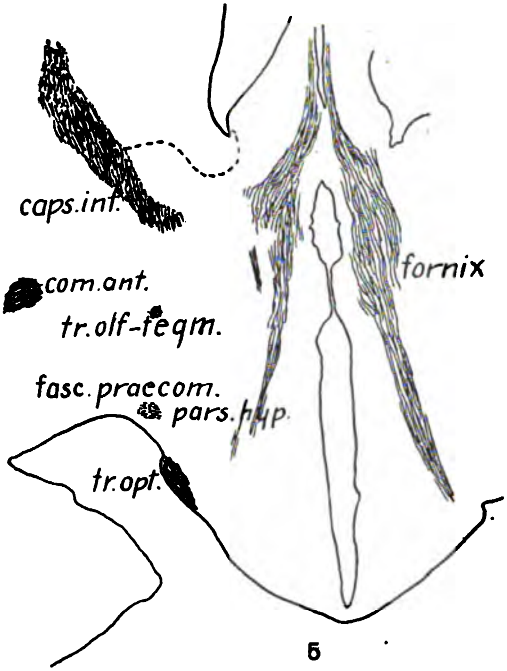
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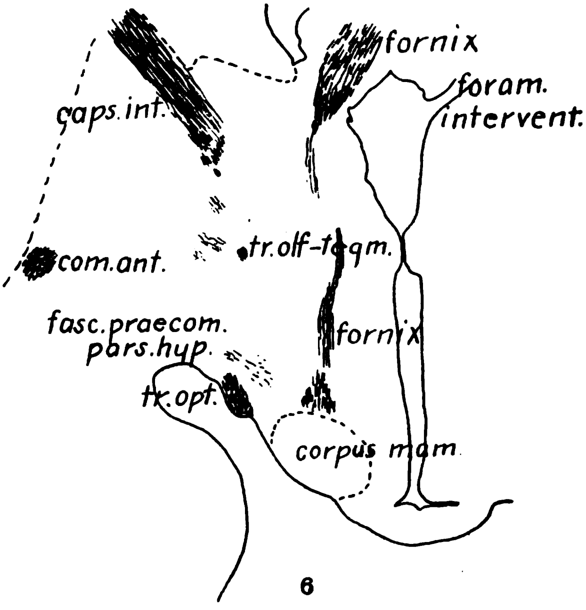
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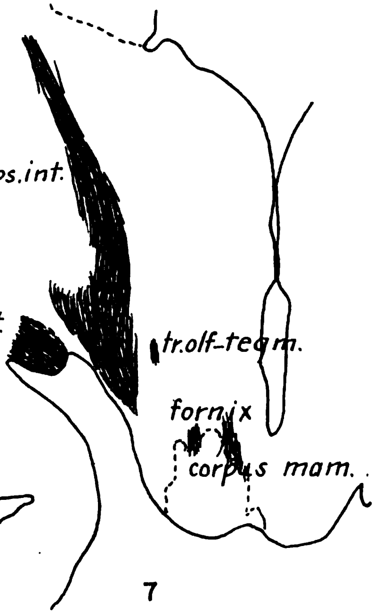
fornix crossing the anterior commissure vertically. On the left side appear a larger and a smaller commissural bundle. The smaller (*bdl. x*) is one of the olfactory bundles of the anterior commissure which crosses the mid-line independently of the main body of the commissure. Crossing these two bundles in a sweeping curve is a small bundle which runs down into the tuber cinereum. When this small bundle is traced rostrad it joins the smaller commissural bundle (fig. 3). Hence, a small bundle separates from the olfactory commissure, passes over the anterior commissure and enters the tuber cinereum. This condition has not before been described so far as the writer is aware.

In the section described there appears beneath the limb of the anterior commissure a fair sized bundle almost round in transverse section (*tr.olf-tegm.*). When this bundle is traced rostrad it is found that it intermingles with the olfactory bundles of the anterior commissure and has a commmon origin with these. This is illustrated in figures 1, 2, and 3. While some fibers may enter these bundles from the olfactory tract, there can be no doubt that most of the fibers are directly related to the olfactory tubercle and adjacent secondary olfactory centers.

The course of this bundle caudal to the level of the anterior commissure is shown in figures 5, 6, 7, 8, 9. The bundle is symmetrical on the two sides, but only the left side is drawn. It runs along the inner face of the internal capsule and descends somewhat into the hypothalamus until it comes to lie between the terminal portion of the fornix and the internal capsule (fig. 7). Beyond the mammillary body it rises again and gains a position dorsal to the medial border of the crus (fig. 8). The bundle then continues between the substantia nigra and the nucleus ruber and divides into several fascicles. These fascicles while continuing spinalward turn rather rapidly dorsad and somewhat laterad. They become imbedded in small masses of cells and are lost just at the upper or anterior border of the pons (fig. 9). One fascicle seems to enter the dorsal part of the pontile gray (fig. 9). One or two fascicles run much farther dorsad than the others, but do not approach so near the brachium pontis.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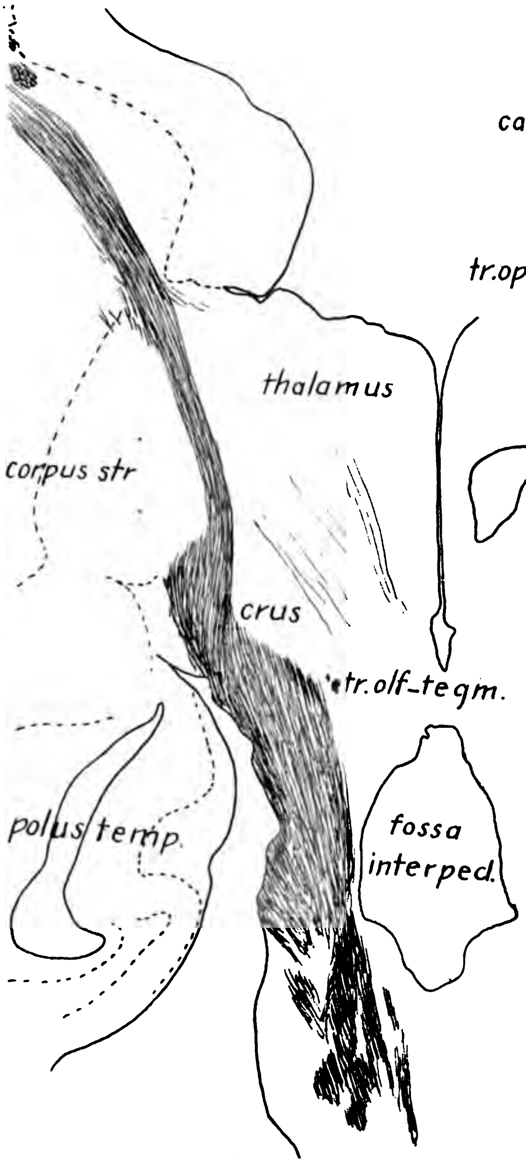
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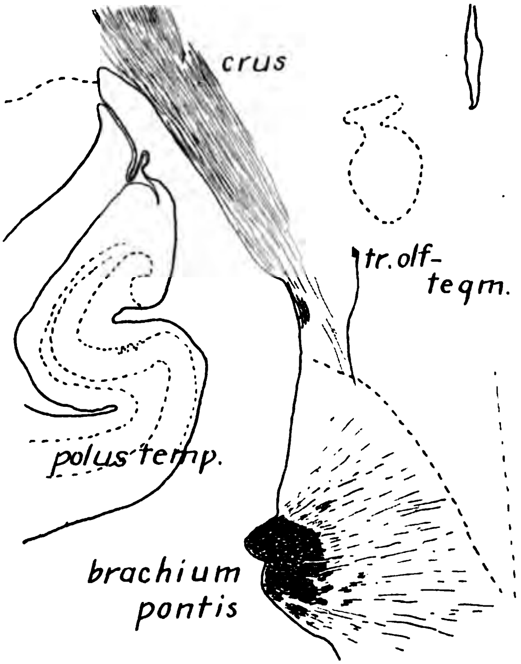

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The bundle described is interpreted as a descending tract from the olfactory centers to the motor-correlation centers in the tegmentum at the level of the isthmus. Such preparations give no direct evidence, of course, as to the actual origin and endings of the fibers. At their rostral end the fibers of this bundle are indistinguishably intermingled with the fibers of the olfactory commissure which we know arise from the olfactory centers. At its caudal end the fibers spread widely in the tegmentum. This renders it very improbable that the fibers arise in any of the sensory nuclei, such as the nucleus of the trigeminus. Its behavior rather suggests the breaking up of a bundle at its place of ending.

From the facts just stated the writer can see no reason for comparing this with the quinto-frontal bundle described by Wallenberg. That bundle is supposed by Edinger (Vorlesungen, 1908) to carry impulses from the sensory nucleus of the trigeminus to the tuberculum olfactorium, which serves as a correlation center for oral and nasal sensory impulses (muzzle-sense). If this were the function of the tuberculum olfactorium, one would expect that in forms like man, in which the olfactory apparatus is reduced and the oral sense is very important, the tubercle would be large relative to the other olfactory centers. Such is not the case. On the other hand the forms in which the tuberculum is large are those in which the olfactory apparatus is very large (Ornithorhynchus, Didelphys, dog).

There is more reason for comparing this with the descending bundle mentioned by Edinger (1908, pp. 123, 286) as arising in the lobus parolfactorius and running through the dorso-lateral part of the oblongata, to the cervical cord, tractus fronto-bulbaris. Edinger's lobus parolfactorius is a large, illy defined region "which includes the whole base of the brain" (p. 252), and there is nothing to indicate from what part of this area the tractus fronto-bulbaris arises. Also, it seems fairly certain that the bundle above described ends largely or wholly at the level of the isthmus. It is quite possible that some of its fibers pass on to the cervical cord and that these constitute the tractus fronto-bulbaris of Edinger.

Figures 2 to 6 show another bundle of considerable interest which is clearly defined in this series. This is the bundle of precommissural fornix fibers which enter the hypothalamus (*fasc. praecom. pars hyp.*). These constitute the most compact bundle in the system of vertical fibers in the "septum" rostral to the anterior commissure (fig. 2). The bundle is conspicuous in the sections back to the level of the optic tract, which it crosses to enter the lateral wall of the tuber cinereum. Whether the fibers reach the mammillary body can not be determined in this specimen.

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THE CELL MASSES IN THE FOREBRAIN OF THE TURTLE, CISTUDO CAROLINA[1]

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SIXTY FIGURES

In continuation of work upon the forebrain, it is the writer's intention to study the origin, growth and differentiation of the general pallium in the series of vertebrates. In doing this it will be necessary to keep constantly in mind the relations of the general pallium to the hippocampal formation, which has been described in earlier papers. The history of the amygdaloid complex and of the pyriform lobe is also intimately concerned. Finally, the position and arrangement of the fiber tracts connecting the hemispheres with the brain stem bear an important relation to the morphology of the cell masses in the forebrain.

In order to be able to compare given brain structures in lower and higher vertebrates it is necessary to have a description of the brain of some intermediate form in which the more common ichthyopsid characters can be recognized and in which at the same time the hippocampus and general pallium are present. For this purpose the brain of the turtle has been chosen and it serves admirably because of the relationship of the chelonia to the ancestors of mammals. The present paper will be confined almost wholly to description, the review of literature and the discussion of hypotheses being deferred to later papers.

METHODS

The results here presented have been obtained chiefly from the study of cell preparations. The results from the study of fiber tracts, although used in connection with this work, will be reported only briefly. The object has been to define the aggregations of neurones which there is reason to think are concerned with specific functions and to determine the position, extent and limits of these cell masses. Criteria depended upon are the position, size, form and grouping of the cells, their relation to fiber tracts and their relation to neighboring cell-masses. The more important aggregations of neurones are either separated from neighboring masses by cell-free zones, or they present a sudden transition to other masses whose cells differ markedly in size, position, staining qualities, etc. Sometimes sulci mark the lines of demarcation. Typical sections have been drawn to show the arrangement of cells in various regions and the lines of demarcation between the aggregations of neurones. To gain an understanding of the morphological relations, dissection and modelling have been practiced. After the various cell masses had been studied and their limits determined, a model was made of the right hemisphere together with a small adjacent part of the diencephalon. In making the plates for this model the limits of the several cell-masses were drawn under the projection microscope. The plates were then cut along these limiting lines but the parts not separated from one another. The model was then piled and fastened together as a whole and the several parts separated at last along the lines of the cuts. In this manner it was possible to pile the model accurately in the form of the control brain and since each part was strongly fastened together there was no distortion of the parts.

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[1] Neurological Studies, University of Minnesota, No. 21, July 20, 1915.

I am indebted to Dr. Wm. F. Allen for the beautiful photographs of this model.

DESCRIPTION

As is well known, the turtle has fairly large hemispheres whose caudal poles lie at the sides of the mesencephalon. The disposition of the hippocampal formation and the olfactory tubercle in the medial wall have been described in a previous paper. Other features in this wall will be taken up in later sections. The lateral wall shows a broad depression where the hemisphere connects with the brain stem which at once suggests comparison with the insular region in the mammalian brain (fig. 1).

The dorsal border of this depression is formed by a low ridge which extends rostrally into the lateral wall of the olfactory bulb. This ridge is traversed by the lateral olfactory tract and is therefore to be compared with the pyriform lobe of the mammalian brain. Near its rostral end it presents an apparent thickening which causes a decided elevation of the surface (figs. 5, 26). The sulcus which bounds the pyriform lobe dorso-laterally varies in depth in different individuals, but is always well marked in its rostral portion. It is the fissura rhinalis.

The insula-like region is clearly seen in the entire brain to be trasversed by bundles of medullated fibers (fig. 1). Sections show these to be a continuation of the crus cerebri (figs. 32, 49), and therefore comparable to the capsula interna. The absence of a cortical layer covering the internal capsule shows that this does not correspond to the insula. It is the corpus striatum which retains a condition analogous to that seen in the fish brain and is not yet covered in by pallium.

Caudal to the striatum there is a well-developed occipito-temporal pole whose presence adds to the similitude of the turtle's brain to that of a mammal. The pyriform lobe appears to blend with the occipital lobe. Along its lower border is a groove which at the rostral end is the sulcus endorhinalis. Behind the striated area a deep groove continues for some distance toward the caudal pole. This groove will be called the fissura amygdaloidea. A part of the area appearing below it in lateral view is occupied by the amygdaloid complex (fig. 5). The reason for calling this the amygdaloid fissure, however, is that it represents the line of infolding of an important body which becomes in mammals a part of the amygdaloid complex.

Rostral to the striated area appears the smooth lateral surface of the tuberculum olfactorium and adjacent olfactory area, including the lateral portion of the homologue of the anterior perforate space. Between this region and the pyriform lobe rostral to the striatum is the sulcus endorhinalis (figs. 19 to 26).

The vault of the hemisphere is occupied, as we shall see, by the general pallium. It is very evident that if the expansion of the general pallium were to push the pyriform lobe ventro-laterally until it was driven down nearly upon the basal surface and until the migrated general pallium itself had covered over the striatum, there would be produced a fairly exact counterpart of the primitive mammalian brain. This is the condition of the brain of an opossum or rabbit, in both of which the lateral (Sylvian) fissure is little deeper than the analogous striatal depression in the turtle's brain. The further expansion of the frontal and caudal poles produces the opercula bounding the lateral fissure.

On the dorsal surface just behind the olfactory peduncle occurs a slight rounded elevation (figs. 3, 25) which forms a part of the pallial thickening to be described below.

On the medial surface of a brain from which the stem has been cut away and the choroid plexus removed (fig. 3) it is seen that a wide chorioid fissure extends caudad in a simple curve from the interventricular foramen into the temporal pole. On removing the medial wall of the hemisphere there are exposed certain ridges which constitute some of the most characteristic features of the reptilian brain (figs. 4, 10). Three main bodies are to be distinguished: a *dorsal ventricular ridge,* the largest and most prominent; beneath it and extending farther rostrad, the *striatum;* and rostrally in the dorsal wall a smaller *pallial thickening.*

Three longitudinal ventricular grooves are to be distinguished: dorsal, middle and ventral. The dorsal ventricular groove is a very deep groove between the dorsal ridge and the pallium (figs. 4, 11 to 22). The middle groove runs beneath the dorsal ridge from the amygdaloid region forward into the olfactory bulb. A bifurcation of the caudal end of this groove and its significance will be mentioned later. The ventral groove is medial to the striatum. It dips very deep into the olfactory tubercle but is shallow rostral and caudal to this level.

The term epistriatum was first used by Edinger to designate a body in the reptilian forebrain to which C. L. Herrick had traced a large part of the olfactory tract. In the 1904 edition of Edinger's textbook this body is labelled epistriatum in figures 117, Varanus; 122 and 123, Schemata; 125b, lizard. This usage is followed by de Lange in a recent paper on Varanus. In Edinger's figure 121 the term epistriatum is applied to an entirely different body in the turtle, the dorsal ventricular ridge of the present paper. In figure 125a, which also represents the turtle, this ridge is called mesostriatum and the term epistriatum is applied to the pallial thickening. Since the structure to which the term epistriatum was first applied does not appear as a special body or ridge in the turtle brain and since the author of the term uses it for at least three different bodies in the reptilian brain, the term will not be used in the present paper. The use of purely descriptive terms will help to avoid confusion.

In the dissected brain or in the model from which the hippocampal formation has been removed (figs. 4, 10) it is seen that the dorsal ventricular ridge bends down into the temporal pole. Here it enters into close relations with the amygdaloid complex which must be discussed later.

FORMATIO OLFACTORIA

The formatio olfactoria is that portion of the forebrain which receives fila olfactoria and contains mitral cells and glomeruli. It forms the rostral part of the olfactory bulb and extends farther caudally on the dorsal than on the ventral surface. Thus the peduncular constriction is placed obliquely, as is evident in figures 3 and 5. The bulb in its rostral part is nearly triangular in cross section (fig. 30). The olfactory nerve divides into distinct ventral and dorsal roots. The ventral spreads upon the dorsal surface of the bulb and enters the brain farther rostrally than does the dorsal root. The ventral root spreads over somewhat more than the lower half of the medial and lateral walls (fig. 30) and the rostral surface. The dorsal root continues over the bulb as a high ridge and spreads out in the dorsal wall in its caudal part. At its greatest extent, near the peduncle, this root extends half way down on the medial and lateral surfaces. The areas in which bundles of fila olfactoria are evident are shown in figures 28, 29, 30. It is certain, however, that many fila extend either in small bundles or singly some distance beyond these limits before ending in relation with neurones of the formatio olfactoria.

The limits of the formatio olfactoria can be determined more accurately by a study of the form, size and grouping of the cells. Throughout all the area where fila olfactoria are visible, the wall of the bulb consists of a thick layer of granule cells adjacent to the ventricle, and a fiber layer of varying thickness in the midst of which are scattered large and small mitral cells. In the areas not covered by bundles of fila olfactoria the mitral cells are less numerous and sometimes absent (figs. 28, 29, 30).

The deep granule cells are very numerous, closely crowded and sometimes arranged in irregular concentric layers. Golgi sections show that they possess two or more long slender dendrites radially placed and that the dendrites interlace with those of the mitral cells in the glomeruli. These cells are therefore true olfactory receptive cells, comparable to the deep cells of the olfactory formation of fishes (Johnston '01). The granule cells form a dense thick layer surrounding the ventricle throughout the whole extent of the bulb (figs. 28, 29, 30) and constitute the greater part of its volume. The groove or constriction which marks the olfactory peduncle follows accurately the caudal border of this granule cell layer. In the lateral wall the pyriform lobe and the olfactory tubercle push forward somewhat and the peduncular groove presents a V-shaped bend forward (fig. 5), but everywhere the groove faithfully indicates the boundary between primary or bulbar structure and the secondary centers or olfactory lobe.

The layer of mitral cells and of small brush cells is not as extensive as that of granule cells. In the rostral half of the bulb this layer is continuous around the periphery of the section, but the cells are less numerous in the lateral and medial angles where the fila olfactoria are not evident. In the caudal part of the bulb the corresponding areas become quite free from mitral cells for some distance (fig. 29, 31). The distribution of the mitral cells is determined by their relation to the fila olfactoria. Golgi preparations show that the dendrites of mitral cells and of the small brush cells penetrate the bundles of fila and ramify richly, thus transforming the bundles into elongated glomeruli or series of glomeruli. A brush often lies on the surface of one of these bundles and its dendrites wrap around and penetrate the bundle. The formation of glomeruli in the course of large bundles of fila is a condition similar to that in Petromyzon described by the writer (1902). In addition to these large glomeruli are numerous smaller ones formed by the dendrites of small brush cells and of granule cells.

The formatio olfactoria consists then of (1) an enormous number of granule cells whose bodies form the dense peri-ventricular layers and whose rather straight dendrites radiate outward to form glomeruli or to mingle with dendrites of other cells in glomeruli; (2) a peripheral layer of mitral cells and small brush cells presenting a great variety of forms, whose dendrites form the large glomeruli by ramifying in the bundles of fila olfactoria; and (3) of the incoming fila olfactoria and the afferent and efferent fibers connecting the bulb with the rest of the brain.

The gross structure known as the olfactory bulb in the turtle consists of formatio olfactoria and nothing else. While the hippocampal formation and the pyriform lobe both push forward into close contact with the olfactory formation, the groove which marks the peduncle corresponds accurately to the line of division in the internal structure. The so-called nucleus olfactorius anterior in this brain is very clearly distinct from the granule cell layer of the olfactory bulb and does not extend forward beyond the peduncular constriction.

TRACTUS OLFACTORIUS

At the olfactory peduncle the fibers of the olfactory tract (figs. 55, 56, 57) have the following disposition. The largest bundles lie on the lateral surface and pass caudad into the pyriform lobe. These fibers arise from the greater part of the lateral and from the dorsal surface of the bulb. On the dorsal surface of the peduncle a large bundle, which has its origin in the dorsal part of the medial wall, crosses obliquely from the medial to the lateral surface and joins the bundle last mentioned. These constitute the lateral olfactory tract. Its further course will be described in connection with the pyriform lobe. Fibers arising from the remaining ventral and medial parts of the bulb collect into a broad sheet ventral to the large bundle first mentioned. This sheet constitutes the medial olfactory tract. It is thick where it abuts upon the large lateral tract but spreads as a very thin sheet over the ventral and part of the medial surface. This tract runs caudad over the ventro-lateral surface of the tuberculum, gradually diverges from the lateral tract and gives diffuse fibers to the tuberculum and the parolfactory area. The medial tract borders the striatal area in front and below as the lateral tract borders that area dorsally.

Two compact terminal bundles of the medial tract are of especial importance. One bends up into the medial wall between the tuberculum and the commissures and is distributed to this region (gyrus subcallosus) and to the hippocampal formation. This is obviously the equivalent of the olfactory tract component in the precommissural fornix system of mammals. The second bundle continues along the lateral border of the medial tract and runs directly caudad to the amygdaloid prominence, where it enters the nucleus of small cells hereafter to be described as the nucleus of the lateral olfactory tract. This bundle runs along with the large bundle which connects the amygdaloid complex with the hippocampus through the precommissural system (olfactory radiations of Zuckerkandl or fiber bundle of the diagonal band of Broca).

CORPUS STRIATUM

Under this name will be described the structures which are homologous with the chief parts of the corpus striatum in human and mammalian brains. Attention has been called to the fibers of the crus cerebri which are seen in a depression on the lateral surface of the telencephalon. A section at the rostral border of this region (figs. 21, 34) shows two great bundles of fibers cut in cross section, the lateral forebrain bundle or crus and the medial forebrain bundle. The fibers of the crus rise in fascicles (internal capsule) scattered through the outer part of the thick latero-basal wall to the dorsal ventricular ridge and pallial thickening. These include somatic sensory fibers and probably also fibers descending to the motor centers. The medial bundle consists of fibers which connect the olfactory centers with the hypothalamus and lower motor correlation centers. From this bundle fibers rise radially into the dense cell mass forming the floor of the ventricle (figs. 21 to 24, 34).

The whole mass in the latero-basal wall is the corpus striatum. It consists of two portions, a lateral much larger part containing large cells and a medial smaller part containing small cells (figs. 21, 22). The coarser fascicles of the crus rise in a curve through the lateral part into the dorsal ventricular ridge and the general pallium (figs. 32, 33). The finer fascicles of the medial bundle rise in almost straight lines radially in the rostral end of the medial part (fig. 34). In man the condensation of the pallial portion of the crus fibers into a plate-like internal capsule has taken place within a lateral large-celled area, the greater part of which is situated external to the capsule and is known as the lentiform nucleus. The remainder of the large cells together with a dense small celled area next the ventricle constitute the caudate nucleus.

In the turtle the large-celled and small-celled areas are clearly distinct from one another. The small-celled area will be called the caudate nucleus, the large-celled area the lentiform nucleus.

The caudate nucleus presents well marked head and tail portions. Far rostrad (fig. 26) this nucleus forms the floor of the ventricle between the ventral and middle ventricular grooves and occupies almost the entire thickness of the latero-basal wall. At its medial border it is continuous with the lateral parolfactory nucleus. This relation has been described for both reptiles and mammals in a previous paper ('13b, p. 389). Its basal surface is largely covered by the medial forebrain bundle from which fascicles enter the caudate. Followed rostrad in transverse sections the caudate is seen to be covered in by the layers of the olfactory tubercle which are continuous with the parolfactory area medially and the pyriform lobe laterally (fig. 27). In these layers are imbedded the olfactory tract fibers as elsewhere described. Continuing forward, the tuberculum and olfactory nuclei increase in thickness and the caudate grows smaller (fig. 28) until just behind the peduncle the caudate disappears. It is this rostral end of the caudate nucleus that the writer identifies with the nucleus olfactorius anterior of Edinger and other authors. Following sections caudad, the sheet of olfactory fibers and cells scarcely disappear from the surface of the caudate until large cells appear in the lateral wall (fig. 26). Beneath the anterior end of the dorsal ventricular ridge this large-celled nucleus rapidly grows larger and the caudate smaller (figs. 22, 21) until the caudate is confined to a part of the floor of the ventricle adjacent to the ventral groove (fig. 20). From this point the tail of the caudate extends caudally to the point where the middle ventricular groove bifurcates (fig. 15, 10). Before this point is reached the caudate is in connection medially with the anterior nucleus region of the thalamus over the crus and the stria medullaris (figs. 17, 18). The caudate then grows thicker and merges insensibly with the small-celled mass of the amygdaloid complex (nucleus of the lateral olfactory tract) (figs. 15, 16). The caudate then disappears from the sections, its place being taken by an area of neuropile. Horizontal and sagittal sections show in this position a cell-free zone bounding the caudate behind, which corresponds to the ventral arm of the middle ventricular groove as it bends down into the temporal horn of the ventricle.

The caudate nucleus throughout its whole extent consists of small cells without regular arrangement. In its head portion it receives fibers from the olfactory tract and the medial forebrain bundle and gives fibers to the latter. In its tail portion are imbedded the stria terminalis and the great lateral olfactory projection tract of Cajal (figs. 48, 49).

A peculiar vesicular structure found in the head of the caudate and in the lateral parolfactory nucleus should be described here. The greater number of these structures are found around the depression of the ventricle which extends down into the tuberculum but some are found also in the lateral part of the caudate not far from the middle ventricular groove. At first sight these structures appear to be clusters of small cells arranged radially about a core somewhat like clusters of grapes about the stem. The clusters are elongated sometimes in the plane of transverse sections, sometimes obliquely to it. In the latter case they may extend through as many as twelve sections ten microns in thickness. When the clusters are cut either across the long axis or lengthwise of it, the core is almost always seen to contain a lumen around which the cells are arranged (fig. 35). The lumen is bounded by a strong limiting membrane and contains more or less of lightly staining material, resembling mucus. Occasionally a flattened nucleus is seen in the lumen.

In the caudate and parolfactory nuclei these vesicles are found only near the ventricle except in the area where these two nuclei meet beneath the ventral ventricular groove. Here they are found farther from the ventricle, even in the deeper layer of the tuberculum olfactorium. In this region these vesicles lie adjacent to or intermingled with the islands of Calleja, to be described below. It was at first thought, indeed, that these were islands of a special type. The vesicular arrangement and other characters of the cells, however, differentiate these structures sharply from the islands of Calleja. The cells are typically columnar and radially arranged around the lumen, the nuclei usually placed near the peripheral end of the cells. The cell-bodies are often filled with small granules unlike the Nissl-bodies of adjacent nerve cells. The nuclei are small, ovoid and dense, resembling those of ependyma cells rather than those of nerve cells. There are often larger cells, obviously nervous, wedged in among or closely applied to the outer surface of the small cells of the vesicles. Although the fascicles of nerve fibers in the caudate often pass close over the surface of these vesicles and sometimes diffuse fibers appear to run ventrad from a vesicle, I have not found in Cajal or Golgi sections fibers arising from the cells of the vesicles.

I suspect that these vesicles are composed chiefly of ependyma cells and that their lumina represent vestiges of ventricular cavity which have been pinched off during development. This might happen owing to the thickening of the caudate and parolfactory nuclei and consequent encroachment upon the ventral part of the ventricle. The fact that these cell clusters are not penetrated by the neuropile (fig. 35) and the fact that no definite fascicles of nerve fibers are found running to or from them, constitute negative evidence as to their nervous function. At the same time, nerve cells are often found closely related to or imbedded in the ependyma and further study will be necessary to determine the origin, character and significance of these structures.

THE LENTIFORM NUCLEUS

At its rostral end this nucleus appears simply as scattered large cells, outside of the small-celled caudate, among which fascicles rise from the crus to the pallial thickening in the rostral part of the roof. Beneath the rostral end of the dorsal ridge this nucleus has grown to a large body in the lateral wall and has reached the ventricle (figs. 22, 23). From this point caudad a peculiar band of thickened ependyma covers the ventricular surface of this nucleus (figs. 15 to 22, 36). This thick band extends some distance below the middle ventricular groove and bends round that groove into its dorsal wall. While the ependyma elsewhere in the lateral ventricle has from one to three rows of nuclei, this band consists of very tall cells and presents as many as seven to nine rows of nuclei. The peripheral processes of these cells are strong and give a prominent striation to the adjoining nucleus (figs. 36, 37). The curved course taken by these processes which come from the dorsal wall of the middle groove (fig. 37) shows that the groove was formed within the ependymal band and near the border of this nucleus. It also shows how the groove has been formed during the development of the individual by the pushing in of the dorsal ridge which forced this border of the nucleus to be bent over. This thickened ependyma extends right back into the dorsal branch of the middle ventricular groove, where it quickly disappears. Throughout its length this thickened band of ependyma covers a nucleus whose cells are distinctly larger and usually much more numerous than those of the adjoining caudate.

In the striatal area this nucleus makes up the whole thickness of the wall and is traversed by the fascicles of the crus as above noted. Toward the caudal border of the striatal depression (figs. 17, 16) the lentiform nucleus becomes covered externally by a layer of small cells which reaches from the pyriform lobe to the amygdaloid complex and will be described below as the nucleus of the lateral olfactory tract. The lentiform nucleus now decreases in volume caudally, contains several dense collections of cells which are closely related to the thickened ependyma of the middle groove (figs. 16, 33), becomes less rich in cells and finally merges with the central core of the dorsal ventricular ridge behind the end of the middle groove (fig.14).

Throughout its whole length the lentiform nucleus is sharply delimited from the pyriform lobe and (except at its caudal end) from the dorsal ventricular ridge, by cell-free zones. It is marked off from the caudate only by a rather sudden change from large to small cells. The thickened ependyma covering its surface is sometimes separated from that covering the caudate by a slight, sharp groove.

Among the fascicles of the internal capsule which come from the sensory bundle of the crus (see later section on the pallium), are found many cells conspicuously larger than the ordinary cells of the lentiform nucleus. The position of these is indicated in figures 18 and 19. They may prove of importance in further comparisons with the mammalian brain.

TUBERCULUM OLFACTORIUM

The tuberculum consists of a cap of two layers of cells covering the basal surface of the caudate nucleus at its rostral end (fig. 27). The outer layer is sparsely filled with small cells and is closely related at its medial border with the medial parolfactory nucleus and at its lateral border with the pyriform lobe at the sulcus endorhinalis (figs. 26, 27). The inner layer has larger cells and is characterized by the grouping into islands which have been described by Calleja and Cajal. These islands are for the most part clusters of the large cells of the deep layer. They contain from four or five to many cells, which present no regularity of arrangement. The cell bodies are pear-shaped or stellate and are largely filled by vesicular nuclei with prominent nucleoli. In these respects they do not differ materially from the cells scattered between the islands. Some of the cells in the islands take a lighter, more transparent and brilliant stain in neutral red than others.

In addition to the islands of large cells there are a number of dense clusters of small cells which are undoubtedly nerve cells. These clusters do not present a core or lumen and the neuropile does not shrink away leaving a clear space around them as happens in the case of the vesicles described in the caudate nucleus. The true islands are confined to the deeper layer of the tuberculum. It must be noted, however, that the tuberculum extends into the medial wall and merges with the medial parolfactory nucleus. The distribution of the islands may arbitrarily be taken as determining the extent of the tuberculum. The greater number of islands is found near the mediobasal angle and they do not extend far laterally. Caudally the islands disappear from the basal surface where the medial forebrain bundle collects (fig. 24) and are found farther caudad in the medial wall.

There is a very close resemblance between the tuberculum olfactorium in the turtle and the 'superficial basal area' described by the writer in selachians (1911). The writer was unwilling at that time to give the name tuberculum olfactorium to this area because it seemed to include parts of the medial and lateral olfactory nuclei and the region corresponding to the anterior perforate space. The close relation of the tuberculum to medial and lateral olfactory nuclei is common throughout vertebrates and I shall present evidence at another time that the tuberculum and anterior perforate space are by no means wholly independent.

ANTERIOR PERFORATE SPACE

The area corresponding to the anterior perforate space of mammals is not sharply marked off in the turtle. It occupies the caudal part of the basal surface of the large rounded prominence rostral to the optic chiasma. The rostral half of this surface shows the characteristics of the olfactory tubercle. The caudal half lacks these peculiarities and is largely filled by the medial forebrain bundle.

DIAGONAL BAND OF BROCA AND GYRUS SUBCALLOSUS

The large basal mass which includes the head of the caudate, the parolfactory nuclei, the tuberculum olfactorium and the anterior perforate space appears on the medial surface as a rounded area (figs. 3, 6) bounded dorsally and caudally by a groove which descends to the basal surface, bends transversely, and disappears. This groove, as the further description will make clear, is the homologue of the fissura prima of His.

The area dorsal to the horizontal limb of this fissure is the primordium hippocampi (Johnston '13 b). The area between the fissure and the lamina terminalis (fig. 6, *g.s.*) is occupied by a cell mass and a fiber bundle both of which are characteristic of this region in all mammals and perhaps in all vertebrates. In a previous paper ('13 b) this cell mass has been included in the description of the medial nucleus parolfactorius in both the turtle and mammals. This was an error. Since this cell mass lies caudal to the fissura prima in mammals, it is outside the limits of the parolfactory area. It may be spoken of as the *nucleus of the diagonal band.* In mammals this consists of a thin plate of closely packed cells which extends from a point rostral to the anterior commissure down in front of the optic chiasma and caudad on the latero-basal surface to the amygdaloid complex. It is very sharply marked in the rabbit and has been figured in the opossum and bat ('13 b, figs. 28, 42). The distinction between this nucleus and the medial parolfactory nucleus is shown in figure 46 of the paper referred to, where this nucleus consists of small cells rostral to the anterior commissure and is not separately labelled. The cell-free zone between this and the medial parolfactory nucleus corresponds to the fissura prima.

In the turtle the same plate of cells is found (figs. 20, 21) occupying the medial surface rostral to the anterior commissure and extending down close in front of the preoptic recess, near the large-celled supraoptic nucleus (fig. 19), and continuing laterally and caudally ectal to the medial forebrain bundle (fig. 18) until it expands into the large anterior nucleus of the amygdaloid complex from which arises the great olfactory projection tract (fig. 17). The relations to the amygdaloid will be further described in the next section. The extreme rostral and upper end of this nucleus is the very dense collection of cells seen near the recessus superior in figures 19 and 39. There is no break in the continuity of the nucleus described but on the basal surface in front of the optic chiasma and lateral to the optic tract, the number of cells is much less than in the medial wall. Throughout the length of the nucleus its cells are small and are somewhat elongated in the direction of the densely packed bundle of fine fibers among which they lie.

The fibers in question enter into the precommissural bundles of the fornix system in the medial wall. They are the olfactory radiations of Zuckerkandl and constitute the chief pathway from the amygdaloid region to the hippocampus. Compare '13 b, p. 410. From the amygdaloid forward along the basal surface these fibers form a low ridge parallel with the optic tract (figs. 1, 8, 19) and in this position the bundle is clearly visible in the entire brain (fig. 2). The bundle is clearly distinct from the medial forebrain bundle which lies ental to this. In front of the optic chiasma the bundle is seen to be composed of two parts, one of which is derived from the medial olfactory tract as above described, while the other bends up into the medial wall, spreads out in the area between the fissura prima and the commissures, and enters the hippocampus (figs. 56, 57). The triangular area between the fissura prima and the commissures in which this bundle spreads out like a fan is the equivalent of the mammalian gyrus subcallosus. Lateral to the optic chiasma this bundle passes close to the supra-optic nucleus and there seems to be a connection between the two which has not yet been carefully studied.

The homology of the area described in this section with the gyrus subcallosus and the diagonal band of the mammalian brain is obvious. Its chief morphological significance is that it represents the persistence of the preoptic and precommissural connection between the medial olfactory area and hippocampus on the one hand and the lateral olfactory area and amygdaloid complex on the other hand. In the ichthyopsid brain where the temporal pole has not yet been formed, the continuity of the corresponding regions is broad and direct. The size and functional importance of this connection in reptiles and mammals is of great significance for the explanation of the evolution of the temporal pole and the general pallium.

LOBUS PYRIFORMIS

As already indicated the pyriform lobe forms a somewhat prominent ridge along the dorso-lateral surface of the hemisphere, extending from the olfactory peduncle into the caudal pole. It is separated from the general pallium dorsally by the fissura rhinalis. Rostrad this fissure runs obliquely mesad and joins the peduncular groove or constriction as it bends down into the medial wall. Caudally the fissura rhinalis presents considerable individual variation in its depth and length. Usually it fades away at about the middle of the hemisphere (fig. 1) so that there is no gross boundary line between the caudal part of the pyriform lobe and the general pallium. In the rostral part of the hemisphere a slight sulcus endorhinalis approximately marks the boundary between the pyriform lobe and the area occupied by the tuberculum and corpus striatum (figs. 22 to 26). The caudal continuation of this sulcus becomes much deeper between the pyriform lobe and the striatal area (fig. 1) and bends down between the striatal area and the amygdaloid prominence. At the point of bending is the deepest part of this groove and from this point the amygdaloid fissure extends caudad as above described.

The pyriform lobe consists of the lateral olfactory tract and the special gray matter accompanying the tract. Sections through the middle or rostral part of the lobe (figs. 19, 25) show that it consists of a superficial fiber layer and a deeper plate of cells and that it is everywhere very clearly and sharply separated from the border of the pallium, the dorsal ventricular ridge and the lenticular nucleus by a cell-free zone. This cell-free zone is more sharply marked than any other in the brain.

The outer surface of the lobe is covered by a continuous sheet of fibers of the lateral olfactory tract. These arise in larger part from the lateral wall of the olfactory bulb and in smaller part from the dorsal and medial wall. The former fibers enter the extreme rostral end of the pyriform lobe on the lateral surface of the peduncle; the latter cross from the medial to the lateral side in the peduncular groove on the dorsal surface and join the former fibers to make up the common tract (figs. 55, 56).

Just behind the peduncular constriction the pyriform lobe appears to thicken rapidly. This thickening forms a conspicuous rounded prominence on the dorso-lateral surface of the hemisphere (figs. 26, 5). The arrangement and relations of the cell masses in this prominence show at once that it is made up of two parts. The surface layer of cells belongs to the pyriform lobe and is no thicker here than elsewhere. The elevation here is due to the deep layer of cells which is the thickened lateral border of the general pallium (figs. 24, 25, 26). This is indeed a lateral extension of the dorsal pallial thickening, which lies beneath the pyriform lobe as the lateral border of the pallium does throughout the rostral two-thirds of the lobe. The pyriform lobe, then, is not actually thickened but is merely bulged out by the thickening of a pallial formation beneath.

The majority of cells in the pyriform lobe are large multipolar cells with large dendrites. In regard to this a comparison of the pyriform lobe with other parts of the forebrain reminds one of the comparison between Deiters' nucleus and adjacent nuclei in the medulla oblongata. The cells are noticeably larger in the rostral half of the lobe than in the caudal, the largest cells and the greatest proportion of large cells being found just behind the prominence above mentioned. In the caudal part the cells become distinctly smaller and where the lobe broadens out to form the lateral wall of the ventricle, the cells take on pyramidal forms much like the cells of the general pallium or the hippocampus.

Throughout the rostral half of the pyriform lobe small cells are almost wholly confined to its ventral portion (figs. 19, 25, 40). These small cells extend somewhat below the sulcus endorhinalis, and the cell-free zone, which accurately marks the boundary of the lobe, meets the surface ventral to the sulcus endorhinalis. This sulcus is therefore actually within the area of the pyriform lobe. At the caudal border of the striatal area the band of small cells along the ventral border of the pyriform lobe spreads ventrad in a broad sheet across the base of the amygdaloid fissure (fig. 5) and expands into a large nucleus of small cells occupying the lateral and rostral part of the amygdaloid prominence (figs. 17, 16). This sheet of small cells is accompanied by a large bundle of fibers from the lateral olfactory tract which ends in this nucleus. This is the nucleus of the lateral olfactory tract (figs. 5, 17).

A further examination of the relations of the small cells in the pyriform lobe shows that essentially two nuclei or two cell groups are to be distinguished. The small cells in the region of the amygdaloid fissure are very clearly separated from the large-celled part of the lobe by a space or cell-free zone (figs. 15, 16, 17). Farther forward although there is no space separating them, the small cells do not mingle with the large cells and the small-celled band becomes continuous with the superficial layer of the tuberculum and through that with the medial olfactory nucleus (fig. 26). The large-celled nucleus, on the other hand, maintains its identity rostrad to the peduncle as already described (fig. 28). Moreover, the location of the small cells below the endorhinal sulcus gains significance from the fact that at the level of the tuberculum the small-celled band spreads ventrally to become continuous with the superficial layer of the tuberculum. The cell-free zone which limits the pyriform lobe internally now becomes continuous with the limiting zone which appears between the caudate sulcus and the layers of the tuberculum (figs. 26, 27).

These relations when summed up with the description that has gone before show that there is a continuous area of small-celled olfactory nuclei comprising the medial parolfactory nucleus, the superficial layer of the tuberculum, the small-celled band of the pyriform lobe, the small-celled nucleus of the amygdaloid complex and the nucleus of the diagonal band. These together constitute a continuous ring which surrounds the crucial-striatal area.

The whole of this ring receives fibers from either the lateral or medial olfactory tract. This continuous complex of small-celled nuclei seems to serve as an intermediate selecting and distributing station through which olfactory impulses are forwarded to the cortical center for sensation, the hippocampus; or to one of the motor correlation centers in the diencephalon, or to centers lower down the brain stem (compare Johnston '15 a). The greater part of the pyriform lobe made up of large cells is probably concerned with olfacto-somatic correlation. Other places for correlation of olfactory and somatic impressions are probably found in the amygdaloid complex and in the hippocampus (subiculum). The distribution of large cells in the pyriform lobe probably gains significance from their relation to the pallial thickening.

The boundary line between the pyriform lobe and the general pallium is less distinct in the caudal part than elsewhere. As the pyriform lobe is followed caudad in sections to the point where it forms the whole thickness of a part of the wall of the ventricle (figs. 13 to 15) the cell-free zone at first continues as a very clear oblique dividing line in this wall (fig. 14). This overlapping of the general pallium by the border of the pyriform lobe is very characteristic. This dorsal border of the lobe rises dorsally in its caudal portion as the model clearly shows (fig. 5). In transverse sections through the region in which the small-celled portion of the pyriform lobe spreads ventrad to merge with the nucleus of the lateral olfactory tract, the cell masses of the dorsal ventricular ridge begin to be connected by a thick curved layer of cells with what appears to be the cell layer of the pyriform lobe in the lateral wall (fig. 15). When this relation is studied in sections farther caudad (figs. 11, 12) it is clearly seen that it is the general pallium which thus enters into continuity with the ventricular ridge. In transverse sections there is no sharp boundary between pyriform lobe and general pallium. It is noticed, however, that there is a narrow portion of the wall adjacent to the dorsal boundary of the lobe in which the cells are placed close to the ventricular surface (fig. 12). The oblique line bounding the lobe dorsally at length gives way to a narrow area in which the cells of the lobe meet those of the pallium above in a confused mass. The ventro-caudal margin is not clear. Sections give the impression that the pyriform lobe merges insensibly with the general pallium in this region. In the model this boundary has been fixed arbitrarily.

THE AMYGDALOID COMPLEX

When the hemisphere of the turtle brain is viewed from the lateral and basal aspects (figs. 1, 2, 5, 6) the region which is occupied in part by the amygdaloid complex appears as a basal prominence similar to the temporal pole of the mammalian brain. It is bounded rostrally by the crucial-striatal area, medially by the optic tract and thalamus and laterally by the amygdaloid fissure. Caudally this prominence passes insensibly into the rounded caudal pole.

Nucleus of the lateral olfactory tract. As above described, a part of the lateral olfactory tract bends down across the amygdaloid fissure just caudal to the striatal area and ends in the lateral border and rostral tip of the amygdaloid prominence (fig. 8). This region is therefore clearly homologous with the nucleus of the lateral olfactory tract in the mammalian brain. This nucleus is composed of small cells which occupy the tip and lateral part of the amygdaloid prominence and extend in a thin layer across the amygdaloid fissure to meet in a thickened border the large-celled nucleus of the pyriform lobe (figs. 16, 17). The two nuclei are separated by a cell-free zone and this condition continues back to the end of the nucleus (fig. 13). At the caudal border of the striatal area the thin layer of small cells is traversed by the fibers of the lateral olfactory tract which bend down into this nucleus, and by numerous fibers from the pyriform lobe which pass over the surface of the nucleus to join the stria medullaris (fig. 56). From the tip of the amygdaloid prominence the fibers of the diagonal band go forward as above described. These are also mingled with the stria medullaris. At the same time the small cells of the diagonal band merge with the small-celled nucleus of the lateral olfactory tract (fig. 17).

Where the thin layer of small cells forms the boundary of the striatal area it covers in the large-celled lateral nucleus of the corpus striatum (nucleus lentiformis) (fig. 16). In transverse sections the nucleus lentiformis is followed caudad for some distance beyond this, always traversed by bundles of crucial fibers which are bending up into the pallium and the dorsal ventricular ridge. The nucleus lentiformis gradually grows smaller and is replaced by the small-celled nucleus which grows thicker caudally and enters into close relations with the caudate nucleus. Indeed, in its rostral portion the entire amygdaloid eminence is filled with small cells, the ventricular portion being the caudate nucleus, the superficial portion being the enlarged continuation of the nucleus of the lateral olfactory tract (fig. 17).

Relations of the caudate nucleus. It has been stated that the caudate ends about opposite the end of the middle ventricular groove and is bounded by the branch of that groove which bends down into the temporal horn of the ventricle. Here the caudate merges with the nucleus of the lateral olfactory tract in a small-celled mass which constitutes the chief part of the amygdaloid complex. In the lizards this mass, or the caudate part of it, is greatly enlarged and forms a prominent lobe or ridge in the ventricle. C. L. Herrick showed that this was an important olfactory center and it is this lobe which was first called epistriatum by Edinger. It was this body whose relations very naturally suggested to Kappers ('08) that the epistriatum of lower vertebrates formed or entered into the amygdaloid complex of mammals. In the turtle this body is simply a part of the amygdaloid complex without hypertrophy. However, if all the other structures to which the term epistriatum has been applied were to be gathered into it, the amygdaloid complex would be a veritable museum!

The caudate nucleus at its rostral end is closely related to the olfactory tubercle and parolfactory area and receives fibers from the medial olfactory tract. At its caudal end it enters into close relations with the nucleus of the lateral olfactory tract and helps to form the olfactory part of the amygdaloid complex.

Medial large-celled nucleus. In sections through the rostral part of the amygdaloid complex the small-celled mass is covered basally by a rounded cap consisting almost wholly of neuropile (figs. 16, 42), only a few medium sized cells being imbedded in it. This neuropile is formed by the interlacing stria medullaris, lateral olfactory tract and olfactory radiations as above described. As the sections are followed caudally the cells in this neuropile increase in number and size until a conspicuous nucleus forms the basal portion of the complex (figs. 15, 43). This is the condition in sections which pass through the caudal border of the stria medullaris. Just caudal to this (figs. 14, 44) the temporal horn of the ventricle penetrates this nucleus. Farther caudally the large cells are all found in the lateral or amygdaloid wall of the ventricle while the hippocampal formation and fimbria appear in the medial wall. The large-celled nucleus lies ventral to the tail of the caudate nucleus. This is the region in which the tail of the caudate merges with the small-celled nucleus of the lateral olfactory tract. Following the sections caudad, the caudate rapidly diminishes and the large-celled nucleus spreads farther dorsad in the large ventricular ridge (figs. 13, 45). At the same time the nucleus of the lateral olfactory tract grows smaller and appears as a rather dense mass of cells near the amygdaloid fissure. Now there appears in the ventral wall between this and the hippocampus an area of scattered cells which on being followed further caudad is found to belong to the general pallium (which see). By the time this condition is reached the caudate has entirely disappeared from the sections and the large-celled nucleus has spread dorsad to the level of the dorsal branch of the middle ventricular groove. Here this nucleus constitutes a ventricular layer of large cells rather closely packed but not arranged in clusters as are the cells of the dorsal part of the dorsal ventricular ridge. Further from the ventricle the lower part of the ridge is filled by the same irregularly placed cells as are found in the dorsal part. Both these and the ventricular layer of large cells merge with the general pallium in the ventral wall. The ventricular layer representing the large-celled nucleus continues over the medial surface of the basal lobe of the ridge to its caudal end.

Further evidence as to the individuality of this nucleus is found in its fiber connections. Only the general disposition of its chief tract will be described here. In the extreme tip of the temporal horn of the ventricle (figs. 10, 13, 14) is seen a small ridge which follows the ventro-rostral border of this nucleus. It is clearly seen in the model and in sections in all planes. This ridge is composed chiefly of fibers which run caudad over the medial surface of this nucleus. In addition to the bundle which forms the ridge there is a thin sheet of fibers in small fascicles covering the whole medial face of this nucleus. All of these fibers gradually disappear as they are traced caudad over this nucleus, in which they have either their origin or ending. Traced rostrad all these fibers pass through the tail of the caudate as a thick bundle. Here the bundle is just lateral (internal) to the stria medullaris as that bundle rises from the nucleus of the lateral olfactory tract to enter the thalamus. The bundle here described now forms the most compact part of the stria terminalis and in addition a large part of its fibers go forward diffusely in the substance of the caudate nucleus. A further discussion of the relations of this bundle will be reserved for a later study of the stria terminalis system. It is evident, however, from the facts given, that what is here described as the medial large-celled nucleus belongs to the amygdaloid complex and the distribution of the stria terminalis bundle confirms the observations as to its extent, based upon the character and grouping of cells.

Dorsal ventricular ridge. Farther caudally in transverse sections the ventricle pushes around both laterally and ventrally until the whole ventricular ridge is seen attached to a pallial wall and no longer to the brain stem (figs. 11, 12). In this pallial wall the extent of the general pallium and hippocampal formation can be made out, at least roughly, as represented in the model. The dense, deep layer of cells of the pallium turns into the ventricular ridge where it is continued by layers of cells lying adjacent to the ventricle. The sparsely scattered cells in the outer part of the pallial wall are continued into the core of the ventricular ridge where they are somewhat more compactly arranged than in the pallium. The section passes through the caudal part of the amygdaloid fissure. It is readily seen that the ventricular ridge corresponds to the external groove and that the fissure is a true fold of the brain wall. Examination of the relations of all parts of the dorsal ridge gives the impression that the ridge is an infolding of the brain wall from this region and that it has pushed forward well toward the rostral end of the hemisphere. Its cell layers come into continuity with those of the pallium here in the amygdaloid fissure and with no other nucleus whatever. They are sharply distinguished from those of the caudate and lenticular nuclei and from the pyriform lobe and are usually separated from these by cell-free zones (figs. 11 to 22). In its rostral part the ridge is sharply separated from all other cell masses and projects freely into the ventricle.

In a transverse section taken at the level of the stria medullaris the cells in this ridge present the arrangement seen in figure 16. All around the ventricular surface are a great number of large and small clusters of cells. Some of these clusters are large, rounded and have a definite outline. Others are very irregular and their outlying cells mingle with the scattered cells between the clusters. The individual cells of the clusters do not differ materially in size from those of the adjacent general pallium but are conspicuously larger than those in any other part of the same section. In the lower part of the ridge, adjacent to the middle ventricular groove, there is a tendency for the cells to be closely aggregated in a large area without special clusters.

The core or central part of the ridge is largely filled by fibers among which are scattered cells. These cells are smaller than those of the clusters. The ventral part of this area is encroached upon by the lentiform nucleus and by the main body of fibers entering or leaving the ridge (figs. 15 to 22). The dorsal part of the central area of the ridge contains a larger number of cells almost evenly scattered. Many of these cells approach in size those of the clusters. This part has almost the appearance of an independent cell-mass and for convenience may be called the core-nucleus. It extends through nearly the whole length of the ridge as a large, somewhat quadrilateral area bounded laterally by the pyriform lobe and centrally by the cell clusters of the ventricular layer Dorsally it is separated from the thickened border of the general pallium by a cell-free zone of fibers and ventrally it is bounded by the chief fiber mass in the center of the ridge. Toward the rostral end of the ridge this body of cells becomes separated into two masses in the transverse section, owing to the general pallium and lentiform nucleus pressing into it from above and below respectively (fig. 22). Caudally this mass of cells becomes less distinct and merges with the superficial cells of the general cortex as noted at first. Throughout the greater part of its length this core-nucleus is penetrated by numerous fibers which pass up from the internal capsule to the thickened lateral border of the general pallium. Although only a few such fibers are seen in Weigert sections, a great number of non-medullated fibers take this course and they are very conspicuous in Cajal preparations.

Comparison of the turtle brain with that of some lower mammals leads to the suggestion that the dorsal ventricular ridge is the homologue of one of the cell masses which constitute the amygdaloid complex in mammals. This is entirely independent of the homology of the 'epistriatum' with the amygdaloid body suggested by Kappers and others. This ventricular ridge is a wholly different structure from the 'epistriatum' of Kappers. This matter will be discussed in a later paper dealing with the amygdaloid complex in the opossum and some mammals. To follow the comparison here would lead beyond the limits of this paper.

The amygdaloid complex in the turtle includes the following structures: the nucleus of the lateral olfactory tract, the tail of the caudate nucleus, the medial large-celled nucleus and probably the dorsal ventricular ridge. The amygdaloid eminence in the temporal pole is encroached upon by both the hippocampus and the general pallium. The amygdaloid complex has related to it the lateral olfactory tract, a bundle of the medial olfactory tract, the bundle of the diagonal band, the stria medullaris, the stria terminalis, fibers of the sensory radiations from the thalamus, an olfacto-hypothalamic projection tract, and an anterior commissure bundle.

The simple enumeration of these tracts shows that while the amygdaloid complex in mammals is a compact collection of cell masses occupying a restricted area in the temporal pole, it is a complex of very diverse elements which have been brought together by mechanical forces and have no primary functional unity.

The extent of the hippocampal formation. The morphological relations of the hippocampal formation have been treated with some fulness in a previous paper ('13 b). There is now to be noticed the boundary between hippocampus and general pallium and the relation of the hippocampal formation to the amygdaloid complex. In the rostral part of the hemisphere the characters shown in figure 47 enable us to fix the boundary of the hippocampus with some degree of accuracy on the basis of cell arrangement. Above the fimbrio-dentate sulcus, which separates the homologue of the fascia dentata from the primordium hippocampi and fimbria, there are first pyramidal cells compactly arranged in a ventricular layer with few peripheral cells. The cells are smaller than the cells in most parts of the general pallium but are neither so small nor so closely crowded as the cells in the deep layers in the middle part of the dorsal pallium in this region. This is the portion which is now generally accepted as the homologue of the fascia dentata of mammals. There is no clear separation or distinction between fascia dentata and hippocampus in this turtle's brain.

Dorsal to this, the cells in the medial wall are less closely arranged, appear larger and are less deeply stained. Many cells here are placed far from the ventricle, even lying in the outer half of the wall. Many cells adjacent to the ventricle are distinctly pyramidal in form and have large, dominating apical dendrites. This loose arrangement of cells rather suddenly gives way to a compact arrangement near the dorso-medial angle of the hemisphere. The dorsal area of compact cells will be described as part of the general pallium. The area of loose cells probably belongs to the hippocampal formation and the transitional region called the subiculum.

As transverse sections are followed caudad the area which is here assigned to hippocampal formation and subiculum grows rapidly wider. There is nowhere a sharp line of demarcation between hippocampus and general pallium, and none is to be expected. There is everywhere noticeable the point at which the deep cell layer becomes abruptly more compact. This point has been taken as the medial boundary of the general pallium in making the model so that what is there shown as hippocampal formation probably includes the equivalent of the subiculum as well (see fig. 6).

As the sections are traced caudad there is a gradual change in the size of the cells. The cells of the hippocampal formation become smaller, those of the dorsal general pallium somewhat larger, until the difference is at first noticeable, then conspicuous. From the level of the commissures caudad the cells of the hippocampus are much smaller than those of the general cortex.

In the occipital and temporal poles of the hemisphere essentially the same characters are found. Near the fimbria border is an area of dense cells in a deep layer. Next to it is an area with cells loosely arranged and many of them near the periphery (fig. 45). In the temporal region this area of loose cells is narrow and is accompanied by an internal sulcus which serves as an additional landmark. The boundary shown in the model made from transverse sections has been carefully checked up by means of horizontal sections and found to be essentially correct.

The fimbria passes over the interventricular foramen and continues back along the border of the hippocampal formation to which the choroid plexus is attached (fig. 57). The bundle decreases in size as it proceeds but is still fairly large at the caudal end of the choroid fissure. Here the fimbria bends around the end of the fissure and spreads in the medial wall of the ventricle medial to the position of the large-celled amygdaloid nucleus. This is sufficient evidence that the hippocampal formation extends around the end of the choroid fissure into the medial wall of the temporal pole and confirms the conclusion based on the study of the cells.

In the temporal pole the hippocampus comes into contact with the general pallium and the large-celled amygdaloid nucleus only, not at all with the nucleus of the lateral olfactory tract or any of the small-celled portion of the amygdaloid complex (fig. 8). The hippocampal formation is intimately related in front with the olfactory bulb and secondary olfactory centers. Caudally it forms the border of the pallium adjacent to the choroid fissure and is bounded on the other side by general pallium. There is no continuity of cell masses between the caudal end of the hippocampus and the caudal part of the secondary olfactory centers. The olfactory apparatus taken as a whole does not form a complete ring following the line of junction of the hemisphere with the brain stem ('limbic lobe'). An opening in this supposed ring occurs between the nucleus of the lateral olfactory tract and the hippocampus.

THE GENERAL PALLIUM

The general pallium occupies the dorsal and caudal walls of the hemisphere and reaches well into the temporal pole. Throughout its whole extent it is interposed between the hippocampus on the one hand and the pyriform lobe and nucleus of the lateral olfactory tract on the other. The pyriform lobe and hippocampus meet one another in front of the rostral end of the general pallium near the olfactory peduncle (fig. 7). The general pallium extends forward slightly further than is shown in the model. The presence of a subiculum between the hippocampus and general pallium and of a definite boundary line between the dorsal border of the pyriform lobe and the general pallium has been described above.

Only the most general features in the structure of the pallium can be described in this paper. By far the greater part of the cell bodies are collected in a dense layer near the ventricle. At the dorso-medial border the cells are mostly pyramidal and there are a few cells scattered in the outer part of the pallium. There are many more of the peripheral cells in the adjacent subiculum.

Farther laterally the layer is more compact and the pyramidal form of the cells less evident.

The lateral border of the pallium presents marked characteristics in the rostral half of the hemisphere. In transverse sections the cell layer has a curve near the lateral border which is slight in the caudal part of the hemisphere (fig. 15), becomes more marked rostrally (fig. 17), and near the rostral end is strongly S-shaped (figs. 18, 19). This, together with the slightly larger size of the cells and their arrangement in a very compact layer, suggests rapid growth and mechanical crumpling of the cell layer. This is the border along which internal capsule fibers enter and leave the pallium and there is doubtless a difference of function between this border and the dorsal or medio-dorsal parts of the general pallium. This lateral border becomes gradually thicker as it is followed rostrad and dips down and becomes more and more overlapped by the pyriform lobe. Here the compact plate of cells becomes a thicker mass which in some sections is broken into several masses (figs. 23, 24). At the rostral end this thickened lateral border produces the pallial thickening mentioned in an earlier section.

Toward the rostral end of the pallium the cell layer becomes much less compact except in the lateral thickened border. The pyramidal cells with prominent apical dendrites are mostly confined to the deep layer and outside of these are many cells of stellate or ovoid form. These cells are present in the whole width of the pallium including the subiculum and the lateral thickening. In some sections these cells seem to constitute an incompletely separate layer. When the pallial thickening begins to form a ridge projecting into the ventricle it is noticed that these cells are more numerous in this part of the pallium than elsewhere (figs. 21, 22) and from this point on rostrad they form a conspicuous outer layer of cells in the pallial thickening (figs. 23, 24, 25, 40).

Near its rostral end the total number of cells in the pallium is greatly increased, the deep layer is much thickened and very dense and the outer layer extends well toward the medial border. Indeed, nearly the whole width of the pallium at its rostral end bears the characteristics of the lateral thick border. In other words, it may be stated that the body recognized in the gross brain as a pallial thickening occupies the rostral end of the general pallial area and extends caudad as a gradually diminishing lateral thick border. The pallial thickening is characterized by the presence of a well-developed peripheral layer of cells and this suggests a special functional significance for this body.

The pallial thickening last described is the most highly specialized part of the general pallium and is farthest removed from the brain stem. The temporal portion of the pallium, adjacent to the amygdaloid fissure, is the most primitive and simple in structure. In the caudal part of the basal surface of the hemisphere, where the temporal and occipital poles may be said to meet, the general pallium presents a somewhat more open arrangement than in most of the pallium (fig. 46). A larger number of cells are placed near the outer surface. From this point rostrad, toward the amygdaloid eminence, there is the line of transition between pallium and hippocampus and an area of pallium in which the cells become more and more irregularly scattered as one follows the sections forward (figs. 45, 44). At its lateral border this pallial area bends inward to become continuous with the cell masses in the dorsal ventricular ridge (figs. 11, 12). As the sections are followed forward it becomes evident that this part of the pallium is related to that basal and caudal part of the ventricular ridge whose ventricular surface is covered by the large-celled nucleus of the amygdaloid complex (fig. 44).

In sections which pass through the caudal part of the pyriform lobe and the deeper and broader part of the amygdaloid fissure (fig. 13), the caudo-lateral prolongation of the nucleus of the lateral olfactory tract appears in the depth of the fissure. Now the general pallial area on the basal surface is bounded by the hippocampus medially and by the nucleus of the lateral olfactory tract laterally. Internally the pallial cells merge with those of the basal lobe of the ventricular ridge. As the sections are followed forward the nucleus of the lateral olfactory tract increases in size while the hippocampal formation disappears from the sections before the tip of the temporal horn of the ventricle is reached (figs. 45, 44). At the same time the tail of the caudate nucleus appears between the dorsal and basal lobes of the ventricular ridge. The large-celled nucleus now surrounds the temporal horn of the ventricle and occupies the pallial surface medial to the nucleus of the lateral olfactory tract. Now the large bundle of the stria terminalis goes upward and forward from the large-celled nucleus, as above described. Finally the large-celled nucleus disappears from the sections at the level of the stria medullaris.

Thus the general pallium extends forward in the basal wall to the tip of the temporal pole. Its cells in this region are of various forms and are irregularly scattered and there is an intimate merging of the pallium with the large-celled nucleus of the amygdaloid complex. There is no area or avenue of continuity of the cell masses of the pallium with those of the brain stem. There is, however, continuity of the pallium, the large-celled amygdaloid nucleus and the lentiform nucleus of the corpus striatum. The lentiform nucleus imbeds the lateral forebrain bundle and is therefore comparable with the somatic area in the lateral wall of the selachian forebrain. In the turtle there is continuity throughout the cell masses related to the afferent and efferent fibers primarily somatic in function in the lateral bundle, or crus. In the fishes the somatic area is continuous with the sensory centers of the thalamus. That this connection has been lost in the turtle may be attributed to the greater development of the hemisphere which has elongated the crus and drawn the lentiform nucleus away from the thalamus.

The general pallial portion of the hemisphere consists therefore of (1) a broad, rather thin wall forming the roof and caudal pole of the hemisphere; (2) bounding this laterally and anteriorly, a pallial thickening which has a peculiar structure; and (3) a temporal area of simpler structure which is intimately connected with the dorsal ventricular ridge. This ventricular ridge might, indeed, logically be listed as a fourth part of the general pallium.

That these four regions are intimately related and constitute one complex area in the forebrain, comparable to the olfactory area with its several nuclei, is attested at once by the distribution of the fibers of the cerebral peduncle or internal capsule. This great bundle, where it is about to pass from the brain stem to the hemisphere (fig. 33), is bounded laterally by the optic tract and dorsally by the stria medullaris and stria terminalis. Medial and medio-ventral to this bundle is a large area of fibers which contains (1) the olfactory projection tract of Cajal ('04, fig. 778) descending from the olfactory portion of the amygdaloid complex, and (2) the medial forebrain bundle which follows the crus into the hemisphere and runs forward in relation to the tuberculum, caudate and other olfactory nuclei.

The great lateral forebrain bundle or crus is composed of two parts, dorsal and ventral. The dorsal part is coarse-fibered and well medullated. It comes from the nucleus rotundus and adjacent cell masses in the dorsal part of the thalamus which correspond to the thalamic sensory nuclei in mammals. The ventral part consists of finer fibers which are well medullated at least in their course through the thalamus and corpus striatum. This bundle bends somewhat ventrad and runs caudad on the ventro-lateral surface of the thalamus and mid-brain until some distance behind the third nerve where it becomes more diffuse and seems to end at least in large part before the isthmus is reached. Without going further into descriptive details it is evident that the dorsal bundle contains the sensory radiations from the thalamus to the hemisphere and that the ventral bundle corresponds at least roughly to the efferent tracts from the hemisphere as we know them in mammals.

These two bundles behave differently in the hemisphere. The dorsal deeper bundle bends up rapidly in larger and smaller fascicles through the lateral part of the striatum and enters the core of the dorsal ventricular ridge. The fascicles spread rather wide apart as they pass through the striatum but are all deep beneath the surface of the brain. Upon reaching the ventricular ridge the fibers enter into the formation of a uniform lace-work in the meshes of which the cells of the core-nucleus are evenly scattered. From this lace-work many fibers proceed into the dorsal pallium. This is the appearance seen in Cajal preparations. In Weigert sections the fibers appear to lose their myelin as they enter the dorsal ridge but a small number of medullated fibers are seen running right through the ridge into the pallium (fig. 32). There is at present insufficient evidence to show what proportion of the non-medullated fibers of the sensory bundle reach the pallium but it is clear that a large part of them do so. When transverse sections are followed from behind forward it is seen that the coarse-fibered sensory bundle all rises to the pallium or the ventricular ridge and disappears from the sections before the rostral end of the ridge is reached. The distribution of fibers to the caudal pole of the pallium is partly through the base of the ventricular ridge and partly by way of the fiber-layer of the pallium itself. Very few medullated fibers reach the caudal pole.

The ventral, fine-fibered bundle spreads laterally in the superficial part of the striatum (nucleus lentiformis) and rises toward the lateral border of the pallium. The fascicles, however, slant forward more as they run dorsally and the whole bundle extends much farther forward than the deeper coarse-fibered bundle. The fascicles break up into diffuse fibers before the pallium is reached and many of the fibers may arise from the lentiform nucleus itself. It is clear, however, that the greater part of these fibers enter into the fiber-layer in which the lateral thickened border of the pallial cell-layer is imbedded. Moreover, the greater part of this bundle is related to the pallial thickening.

In sagittal sections (fig. 51) fascicles from the superficial bundle are traced directly up to the pallial thickening through the rostral part of the striatum, and also around the rostral end of the striatum through the cell-free zone between the striatum and the pyriform lobe. It is interesting to note in sagittal sections that medullated fibers enter the pallium and the ventricular ridge only in the rostral part (half or more) of each. The bundles which rise from the crus into the core of the ventricular ridge lose their myelin or disperse so that they can not be traced farther as fascicles.

In Chrysemys marginata the ventricular ridge is smaller than in Cistudo and does not extend as far forward. The pallila thickening on the other hand is relatively more extensive. In this form the direct course of the superficial fibers of the crus up to the pallial thickening is much more clear than in Cistudo.

It is hoped to test the disposition of these fibers experimentally. If the above conclusions drawn from the study of sections prove to be correct, it is evident that the pallial thickening must be regarded as the chief source of the fibers descending from the pallium to the brain stem. It should be noted that the pallial thickening occupies the same position in the hemisphere as that occupied by the motor area in the lower mammals, except that there is no appreciable frontal association area seen in front of it. Also, the descending fibers lie in front of the sensory radiations as they do in the mammalian internal capsule. It seems probable that the pallial thickening is chiefly motor (efferent) in function and that the ventricular ridge and a large part of the general pallium are sensory and associational. However, it is probable from my present results that the sensory and motor areas are not wholly independent. Also the whole subject of localization within the sensory field is untouched in the present paper.

SUMMARY AND COMMENTS

The advantage offered by the turtle brain for the study of the evolution of the mammalian cerebral hemisphere is that the turtle occupies such an intermediate position between fishes and mammals that it is possible from the standpoint of the turtle brain to recognize the homologous structures in all three groups and to explain the changes which have taken place from the brain of the fish to that of the turtle and from the brain of the turtle to that of the marsupials and mammals. This is due to the fact that among living reptiles the turtles are most nearly related to the ancestors of mammals and still possess a relatively simple and slightly specialized brain. In some points there would be great advantage from the study of the dipnoan brain, to bridge the gap between the fishes and reptiles. This gap is much wider than that between the reptiles and mammals, since neither the specialized fishes nor the amphibians stand in the line of descent of reptiles and mammals. The dipnoans, or some of them, do stand in this line of descent and the evolution of the brain must be traced from the selachians, through the dipnoans, reptiles and marsupials to the mammals.

The main purpose of the present paper is merely to define the morphological relations of the chief cell masses in the brain of the turtle in order that these may be used in future studies as points of reference in comparing ichthyopsid, reptilian and mammalian brains. This is done in the belief that a satisfactory account of the mammalian and human brain must eventually include an account of the origin and evolution of the several structures together with an explanation of existing structural relationships and the course of their evolution on the basis of environment, habits of life and the function of the several parts.

The olfactory area

Upon comparing the secondary olfactory centers of the hinge turtle with those of fishes, the only very noteworthy fact is the appearance in the turtle of a nucleus of the lateral olfactory tract behind the area occupied by the lateral forebrain bundle. This is a distinctly mammalian character. In the selachian (Johnston '11 a) the lateral forebrain bundle is imbedded in the somatic area in the lateral wall of the slender portion of the forebrain known to many authors until recently as the 'praethalamus,' and the lateral olfactory area lies wholly in front of this. The relations are shown in figure 59, in which are roughly indicated the areas from which alone the pyriform lobe and the nucleus of the lateral olfactory tract may have been developed. In the turtle these areas have moved far backward on the lateral surface and the mass which imbeds the lateral forebrain bundle appears as an island surrounded by olfactory nuclei,—the striatal area surrounded by the pyriform lobe, nucleus of the lateral olfactory tract, the diagonal band, the parolfactory nuclei and the tuberculum. This condition, which is so suggestive of, but not wholly similar to, the mammalian condition, has come about by a spreading and migration caudad of the lateral olfactory area, chiefly brought about by the great expansion of the dorsal pallium. The expansion of the pallium was accompanied by a great thickening of the lateral bundle and of the cell mass in which it was imbedded. The stretching of the lateral olfactory area accompanying the expansion of the pallium was followed by a collecting together of the olfactory centers into a ring around the striatal area. The striatum then, thickening and bulging outward, came to have its surface freed from the olfactory layer. That a complete ring of olfactory centers was formed around the striatal area was due to the connection which the medial olfactory tract retained with the nucleus of the lateral olfactory tract (fig. 56). This connection of the lateral olfactory nucleus with the medial tract and diagonal band has produced the characteristic bending of a part of the lateral olfactory tract to reach its nucleus in the amygdaloid region (fig. 60).

We have now to note how this condition in the turtle differs from that in the mammal. Although the gross appearance is much the same there is one essential difference, namely, that the striatal area has received a covering of general pallium, converting it into the insula. This has come about by the further expansion of the pallium which has pushed the pyriform lobe down over the striatal area so that in mammals it runs along the ventral border of the insula. Now a part of the lateral olfactory tract sweeps round in a curve to reach its nucleus in the tip of the temporal pole just as in the turtle. There is this difference, however, that the concavity of that sweeping curve is filled not by the striatal area but only by the tuberculum and the anterior perforate space.

The striatal area has thus been the scene of two important migrations of brain substance in the history of vertebrates. Between the fishes and reptiles the olfactory area has spread over the striatum and left its surface free again in the turtle. Between the turtle and mammals the general pallium has spread down over the striatum and left it covered by the insular cortex.

The hippocampal formation

With the expansion of the lateral brain vesicle and the formation of a caudal pole the massive hippocampal primordium in the roof of the selachian forebrain becomes spread out into a thin medial wall of the expanding vesicle. At the same time a part of the choroid roof of the median ventricle is carried out in the medial wall of the lateral ventricle and forms the choroid plexus extending from the interventricular foramen to the temporal pole. The relations of the hippocampus to the secondary olfactory centers in the medial wall have been fully discussed in previous papers and the history of the hippocampus above the reptiles is well known from the work of Elliot Smith and others.

The general pallium

The force which has brought about the changes in the olfactory centers mentioned above is the expansion of the general pallium. This is derived from the anterior end of the somatic sensory column (Johnston '10 c and '11 a) which early in vertebrate evolution extended up into the roof of the forebrain between the lateral olfactory area and the hippocampal primordium. This mass of cells received ascending fibers from the sensory centers in the thalamus and rapidly developed into an important collection of sensory and correlating centers for somatic relations. These centers proved of incomparable value to those vertebrates in which they were the more highly developed, through enabling them to extend the range of environment to which they could adapt themselves.

This expanding general pallium was located between the olfactory area and the hippocampus and was connected with its fellow of the opposite hemisphere by a commissure (corpus callosum) running through the substance of the hippocampus and in close relation to the hippocampal commissure. Under these conditions the expansion of the general pallium brought about great changes in the adjacent structures, as already noted. The first effect was an expansion distinctly in the dorsal or pallial portion of such a lateral forebrain vesicle as exists in the simpler selachian brains. This tended to produce a caudal pole pushing back over the stem or 'prethalamic' portion. To understand the formation of the caudal pole it is essential to see that the expansion began in this dorsal region and proceeded in the direction eventually assumed by the choroid fissure and the hippocampus. From such a beginning the stretching of the hippocampus and pyriform lobe and the migration of the olfactory area over the striatum all are readily understood. The further expansion of the pallium and the differentiation of special centers in it must be the subject of future work for which the present is preparatory.

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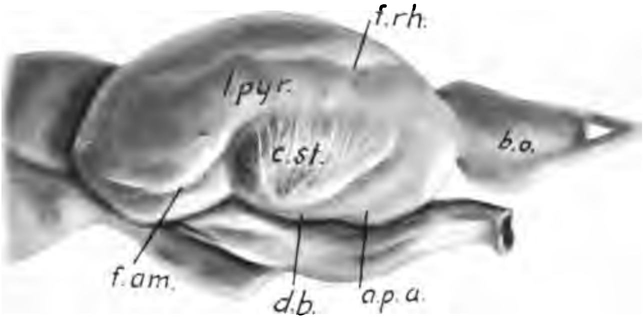
REFERENCE LETTERS

a.p., area parolfactoria
a.p.a., area perforata anterior
b.o., bulbus olfactorius
c.a., commissura anterior
c.h., commissura hippocampi
c.i., capsula interna
ch.op., chiasma opticum
c.post., commissura posterior
c.p.a., commissura pallii anterior
crus, crus cerebri or lateral forebrain bundle
c.st., corpus striatum
d.b., diagonal band of Broca
dec.po., decussatio postoptica
d.v.r., dorsal ventricular ridge
f.am., fissura amygdaloidea
fasc.m., fasciculus marginalis
f.chor., fissura chorioidea
fi., fimbria
f.pc., fasciculus praecommissuralis
f.o., formatio olfactoria
f.p., fissura prima
for.i., foramen interventriculare
f.rh., fissura rhinalis
gl., olfactory glomerulus
g.p., general pallium
gr., granule cells and layer
g.s., gyrus subcallosus
h., hippocampus
hy., hypothalamus
i.C., islands of Calleja
l.pyr., lobus pyriformis
l.t., lamina terminalis
m.fb.bdl., medial forebrain bundle
mi., mitral cells and layer
n.c., nucleus caudatus
n.d.b., nucleus of the diagonal band
n.l., nucleus lentiformis
n.med.a., large-celled medial nucleus of the amygdaloid complex
n.o., nervus olfactorius
n.o.a., nucleus olfactorius anterior
n.p.l., nucleus parolfactorius lateralis
n.p.m., nucleus parolfactorius medialis
n.rot., nucleus rotundus
n.sup., nucleus supraopticus
n.tr.olf.lat., nucleus of the lateral olfactory tract
olf.proj., olfactory projection tract
pa., pallium
pa.th., pallial thickening
p.c., layer of peripheral cells in the pallium
p.h., primordium hippocampi
p.o. pedunculus olfactorius
r.p., recessus praeopticus
r.n., recessus neuroporicus
r.s., recessus superior
s.en., sulcus endorhinalis
s.f-d., sulcus fimbrio-dentatus
s.l., sulcus limitans hippocampi
s.m., stria medullaris
s.p.a., substantia perforata anterior
s.t., stria terminalis
sub., subiculum cornu ammonis
s.v.d., dorsal ventricular sulcus
s.v.m., middle ventricular sulcus
s.v.v., ventral ventricular sulcus
thal., thalamus
t.o., tuberculum olfactorium
t.p., temporal pole
t., tectum mesencephali
tr.d.b., tract of the diagonal band
tr.olf.lat., lateral olfactory tract
tr.olf., tractus olfactorius
tr.olf.med., medial olfactory tract
tr.op., tractus opticus
v.III., third ventricle
v.l., ventriculus lateralis

Fig. 1 Lateral surface of forebrain. Chief features described in text. The fibers in the striatal area are readily seen both in the fresh brain and in brains prepared in bichromate. These fibers are equivalent to the mammalian internal capsule, but the cortical covering of the insula is wanting. The pyriform lobe shows some cross ridges which probably correspond to bundles of internal capsule fibers and thickenings of the lateral border of the pallium. The amygdaloid fissure was especially well developed in this brain. The whole brain never shows the rostral end of the sulcus endorhinalis as well as sections do.



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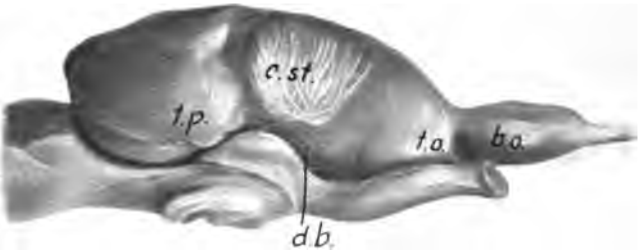

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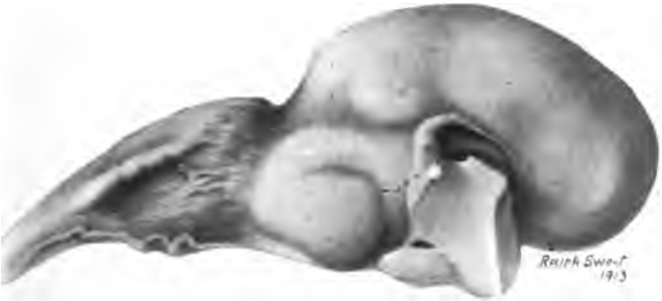

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Fig. 2 A ventral view of the same brain as in figure 1. The diagonal band and the fibers imbedded in it are readily visible in the entire brain, although the fiber-bundles are much more delicate than those of the internal capsule.

Fig. 3 A medial view of another specimen. (From Johnston '13 b, fig. 20.) The fissura prima curves forward over the parolfactory area. The fimbrio-dentate sulcus runs longitudinally above it. The body between is the primordium hippocampi. Between the fissura prima and the lamina terminalis the diagonal band rises apparently to join the hippocampal primordium directly. The broken line indicates the boundary of the primordium as determined by internal structure. The portion of the diagonal band seen on this surface of the brain is the homologue of the gyrus subcallosus. This figure shows the prominence of the rostral end of the pallium due to the pallial thickening.

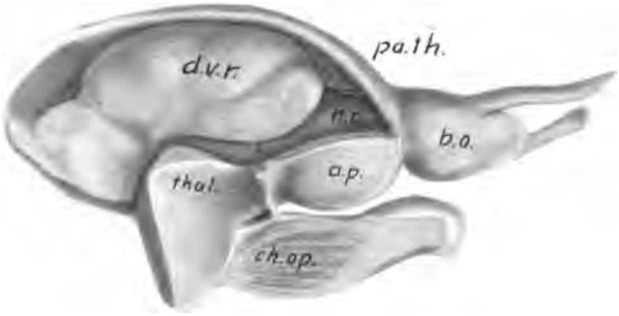
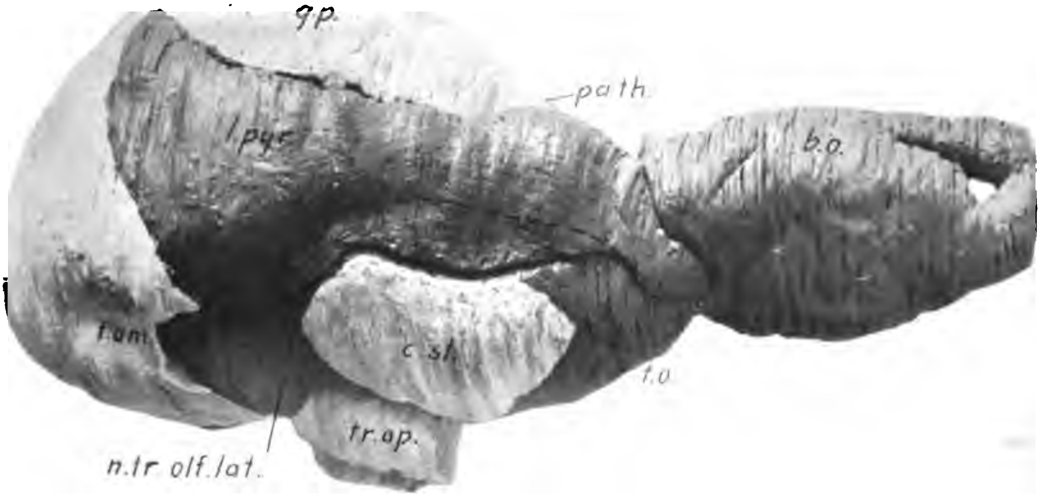


Fig. 4 A forebrain from which the entire medial wall of the pallium has been cut away, seen from the medial direction. The large dorsal ventricular ridge is divided into a chief lobe and a basal lobe by a shallow groove. The bifurcation of the middle ventricular groove is not well shown. One branch descends into the temporal horn of the ventricle; the other runs for a short distance in the bottom of the shallow groove on the dorsal ridge. Compare figure 10. This brain shows an unusual prolongation of the basal part of the dorsal ridge into the caudal pole. The part of the striatum seen here is the head and tail of the caudate nucleus.

Figs. 5 to 10 These are photographs of a model of the right half of the forebrain made by the method of plate reconstruction. The method of building up and dissecting the model is explained in the text. Before photographing, the outer surface of the model was lightly smoothed and then painted in two colors. All the olfactory centers including the hippocampus were painted a light yellow-green. The somatic structures, striatum and general pallium, were painted light blue. In the photographs all the olfactory centers have a dark tone. The ventricular surfaces and the cut surfaces have received no treatment whatever. The hypothalmic region was not completed.

Fig. 5 Lateral surface of the model. The dorsal border of the pyriform lobe is sharply marked through its whole length. The caudal border is only arbitrarily fixed. A broken line marks the limit between the large-celled and small-celled parts of the pyriform lobe. The olfactory part of the amygdaloid eminence appears depressed owing to its color and to its being in shadow. Compare figure 8. The structures surrounding the striatal area constitute a continuous ring of small-celled olfactory nuclei.

Fig. 6 Medial view of the model. The lip of the choroid fissure and the lamina terminalis are painted white. The model is cut between the hippocampal commissure and the anterior commissure, so that the commissure may be removed with the whole hippocampal formation. A broken line indicates roughly the boundary between the hippocampus proper and the subiculum. Probably too large an area is included in the subiculum at the anterior end. The somatic pallium should have been carried farther forward here in constructing the model. Note the oblique position of the peduncular constriction. Note also the fissura prima, the fimbrio-dentate sulcus and the moulding of the caudal pole around the thalamus and midbrain.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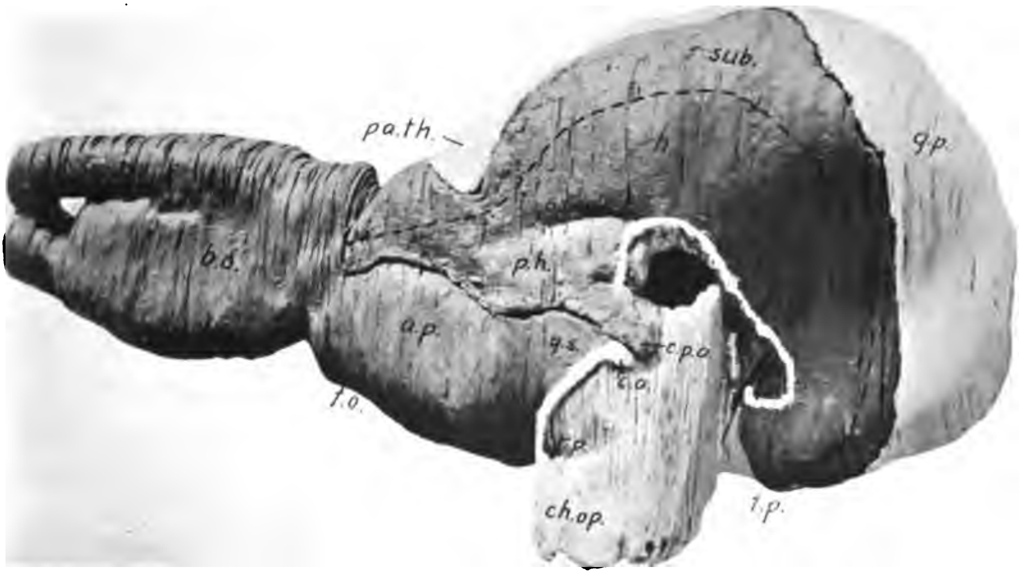
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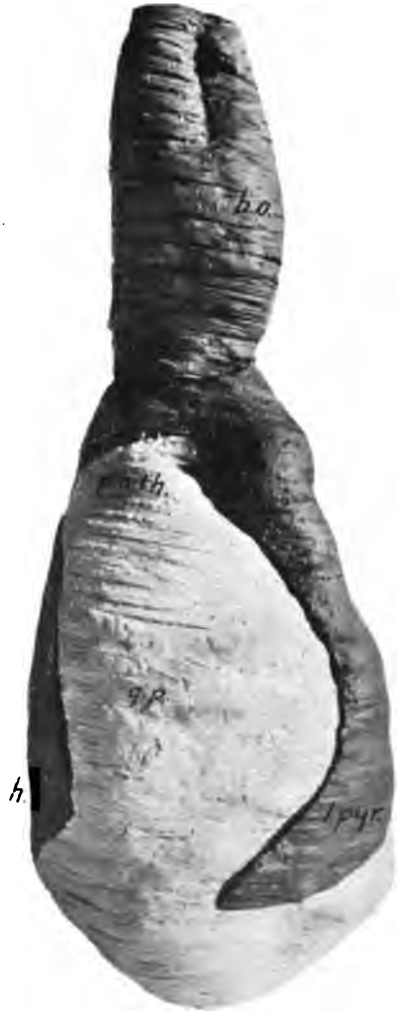


Fig. 7 Dorsal view of the model. This requires no comment except to point out that the general pallium should have been continued somewhat farther forward at the expense of the subiculum cornu ammonis.

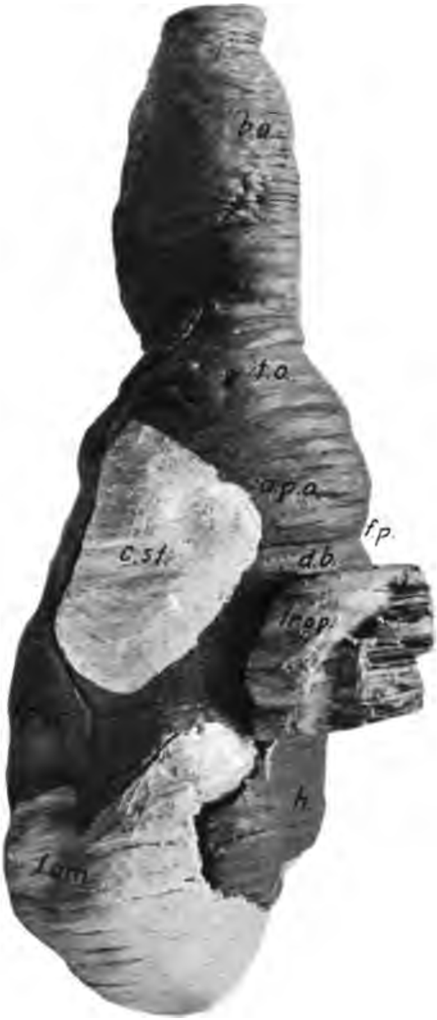


Fig. 8 Ventral view of the model. This view shows well how the striatal area is completely surrounded by olfactory centers. It also shows that the general pallium extends into the amygdaloid prominence of the temporal pole so as to intervene between the hippocampus and the secondary olfactory nuclei. The temporal pole of the hippocampal formation has no direct relation with the secondary olfactory nuclei on the basal aspect of the brain.

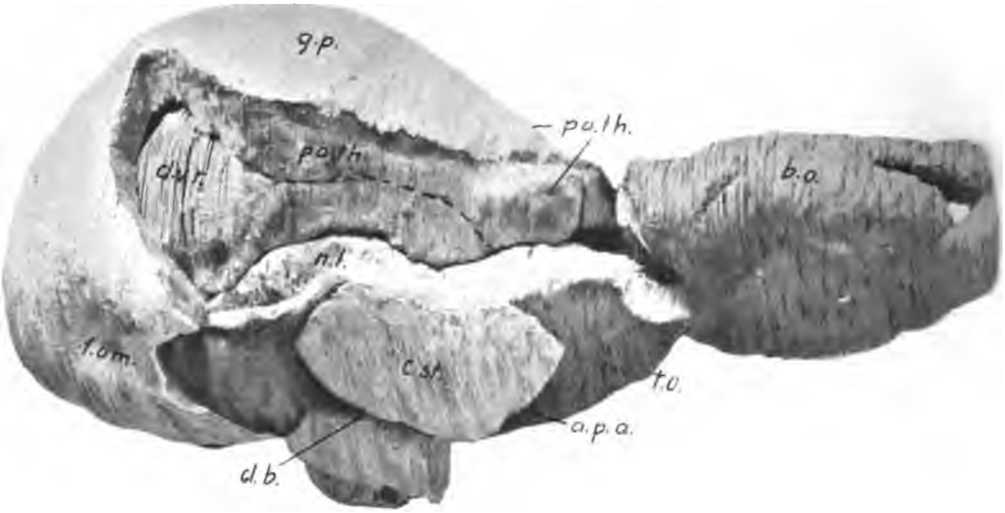


Fig. 9 Lateral view of the model with the pyriform lobe removed. At both its rostral and caudal ends the pyriform lobe forms the whole thickness of the wall over small areas, so that the ventricle is opened in this photograph. Through the greater part of its length the pyriform lobe overlies the thickened lateral border of the pallium and the dorsal ventricular ridge. A broken line marks the boundary between the pallium and this ridge. Along this line a cell-free zone separates the pallium from the ridge but the two can not be dissected apart owing to the continuity of their cell masses in the region of the amygdaloid fissure. The thickening of the lateral border of the pallium which causes the bulging out of the pyriform lobe near its rostral end is clearly seen in this photograph.

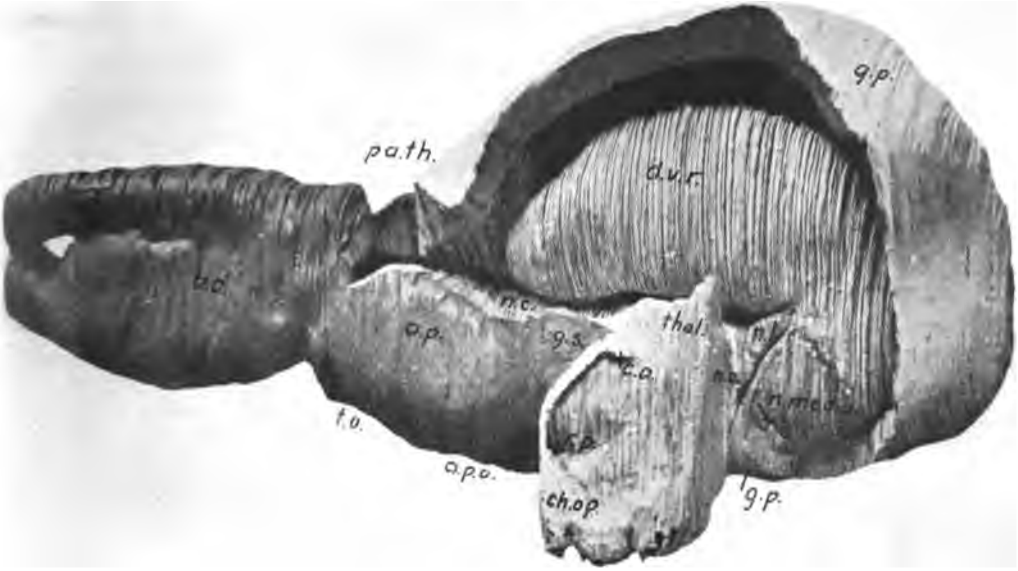


Fig. 10 Medial view of the model with the hippocampus removed. The pallial thickening is perhaps better seen here than in the dissected brain (fig. 4). That part of the thalamus which appears behind the anterior commissure is continuous laterally with the tail of the caudate. The ventricular ridge and general pallium are separated from the striatum by a cut which follows the middle ventricular groove to the end of its dorsal branch, and by a cut which runs diagonally downward and forward from the end of the first one and disappears behind the hypothalamus in this view. The bifurcation of the middle ventricular groove is seen just behind the upper angle of the thalamus. From this point the temporal branch descends into the temporal horn of the ventricle. Its course forms a letter X with the second cut above described. Following the course of this groove is the small ridge described in the text as imbedding part of the stria terminalis related to the basal lobe of the ventricular ridge. From this ridge the bundle goes forward in the caudate. The tail of the caudate barely appears in this figure behind the thalamus (n. c.) and bounded by the temporal branch of the middle groove. The thickening and fusion of the caudate with the nucleus of the lateral olfactory tract in the amygdaloid complex lies just lateral to this point. It is in this region that a great hypertrophy of the common caudate-amygdaloid mass produces the 'epistriatum' in the lizards. The triangular area between the two cuts above described belongs to the lentiform nucleus. Note that the general pallium is continuous with the lentiform nucleus through the basal lobe of the ventricular ridge (medial nucleus of amygdaloid complex).

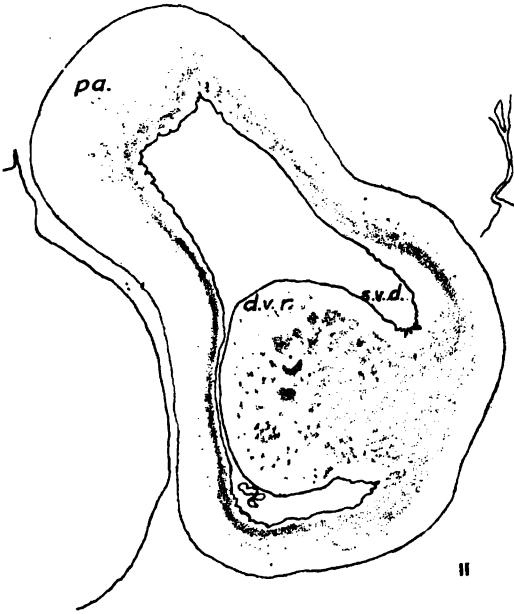
Figs. 11 to 30 Transverse sections of the right hemisphere drawn from sections stained to demonstrate the size and grouping of the cell bodies. The contrasts and lines of separation are not exaggerated. Magnification, 17.5 diameters. The position of each of these sections is indicated in figure 58.

Fig. 11 Section through the base of the dorsal ridge. The dense layer in the medial wall of the pallium is in part hippocampus. The rest of the section belongs to general pallium, with which the ridge is in connection.

Fig. 12 Section a short distance behind the choroid fissure. The dense pallium in the medial wall is hippocampal. The dorsal ridge appears as an infolding of the general pallium. The pyriform lobe presents a confused arrangement of its cells.

Fig. 13 Section at the caudal border of the choroid fissure and the base of the amygdaloid fissure. Hippocampal formation appears both above and below the choroid fissure. The large-celled nucleus appears on the ventricular surface of the basal lobe of the great ventricular ridge. The nucleus ot the lateral olfactory tract appears near the base of the amygdaloid fissure. Between them the general pallium is continuous with the central core of the ventricular ridge. Note the small ridge below, which contains the stria terminalis bundle. From this level forward the subiculum cornu ammonis is a conspicuous feature.

Fig. 14 Section through the extreme rostral end of the temporal horn of the ventricle. Hippocampal formation has disappeared below the choroid fissure. The large-celled nucleus surrounds this part of the ventricle. The nucleus of the lateral olfactory tract has grown larger and the caudate nucleus appears and is fused with it. The nucleus lentiformis begins to take shape.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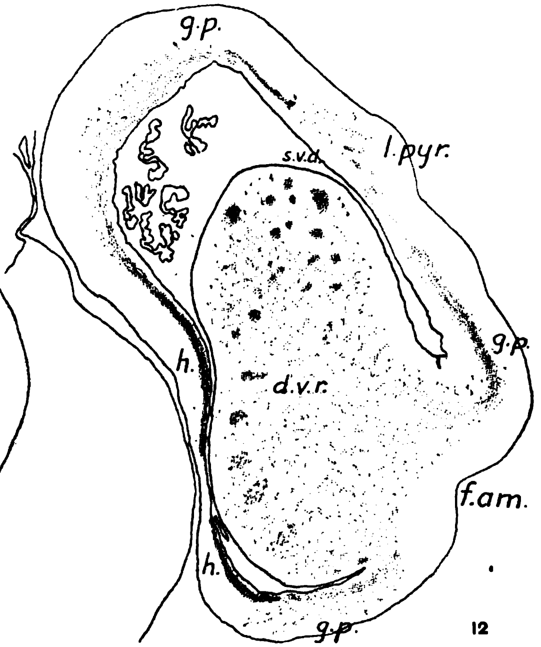
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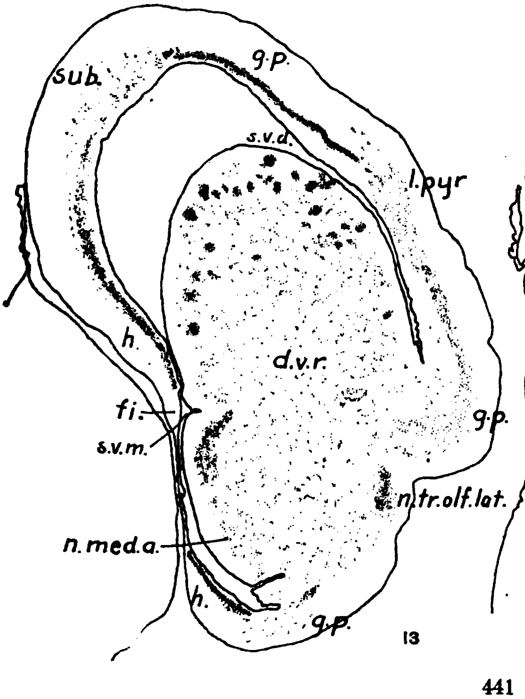
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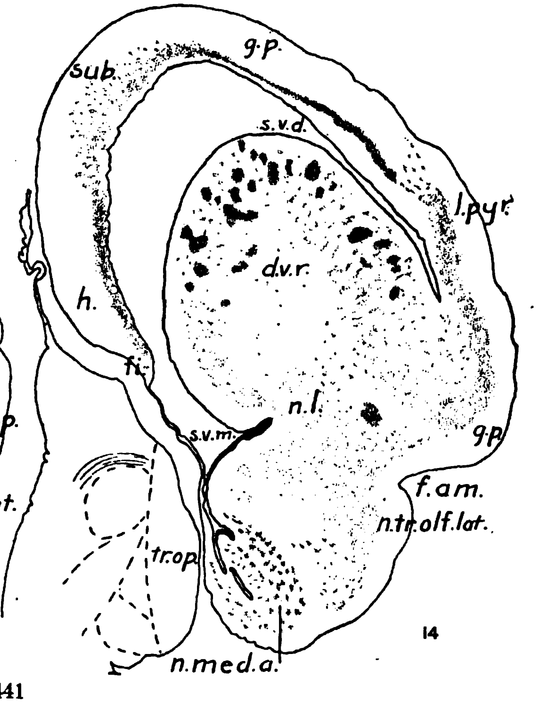
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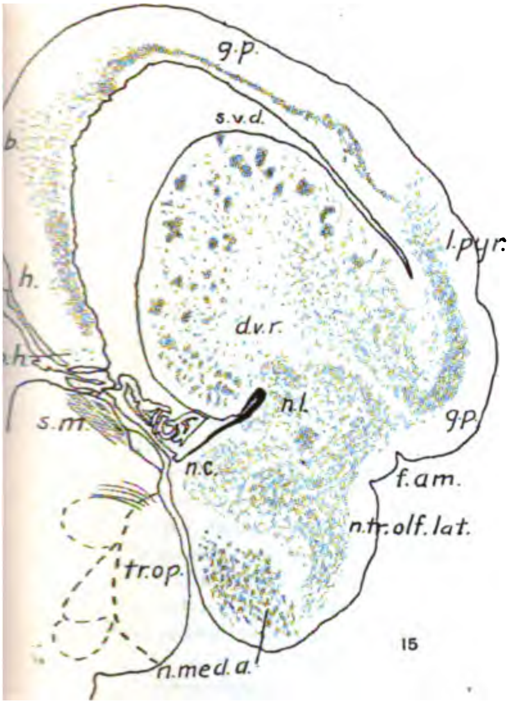
Fig. 15 Section at the base of the amygdaloid fissure. The general pallium still makes connection with the ventricular ridge and intervenes between the pyriform lobe and the nucleus of the lateral olfactory tract. This last is now larger and merges broadly with the caudate. The lentiform nucleus shows some dense collections of cells.

Fig. 16 Section through the stria medullaris. This is about the largest and densest part of the small-celled area of the amygdaloid complex, consisting of the caudate and nucleus of the lateral olfactory tract. The great enlargement of this body produces the 'epistriatum' in lizards. The olfactory projection tract and part of the stria terminalis are related to this (compare fig. 49) while the stria medullaris enters the neuropile surrounding the rostral end of the medial nucleus. Note the rapid thickening of the lateral border of the dorsal pallium in figures 15 and 16. An angle of the general pallium connected with the ventricular ridge still intervenes between the pyriform lobe and the nucleus of the lateral olfactory tract.

Fig. 17 Section through the extreme tip of the temporal pole or amygdaloid prominence and through the caudal part of the junction of thalamus and striatum. The nucleus lentiformis is larger and the nucleus of the lateral olfactory tract appears divided. The upper portion will in the next figure join the pyriform lobe. The core-nucleus of the ventricular ridge is now well defined. Note the olfactory projection tract curving over the crus.

Fig. 18 Section through the commissures just rostral to the inter-ventricular foramen. The crus bends laterad into the lentiform nucleus. It separates the caudate from the nucleus of the diagonal band which is a continuation of the nucleus of the lateral olfactory tract. Note the continuity of the caudate with the nucleus anterior region of the thalamus in figures 17 and 18. Note also the rapid enlargement of the primordium hippocampi in these figures as it approaches the level of the commissure. For the disposition of the commissures compare figures 32, 48 and 52.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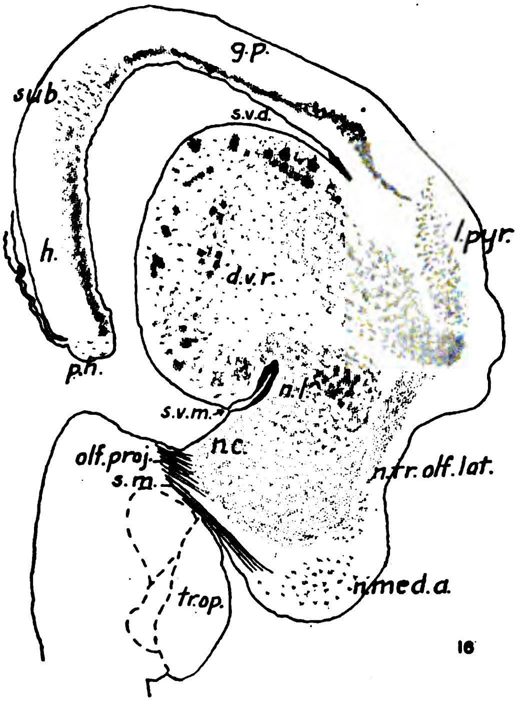
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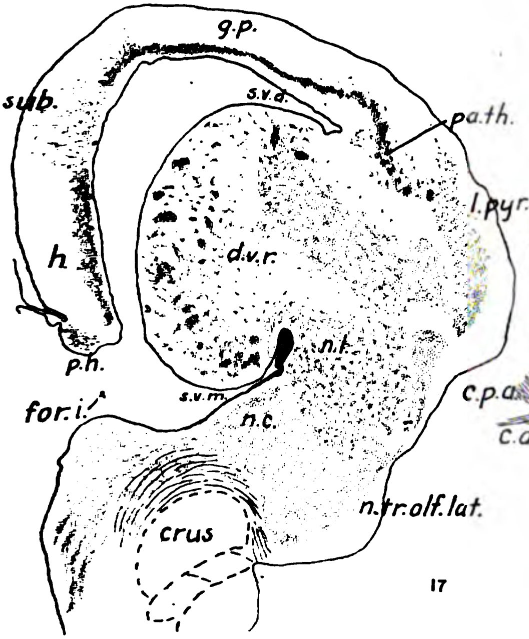
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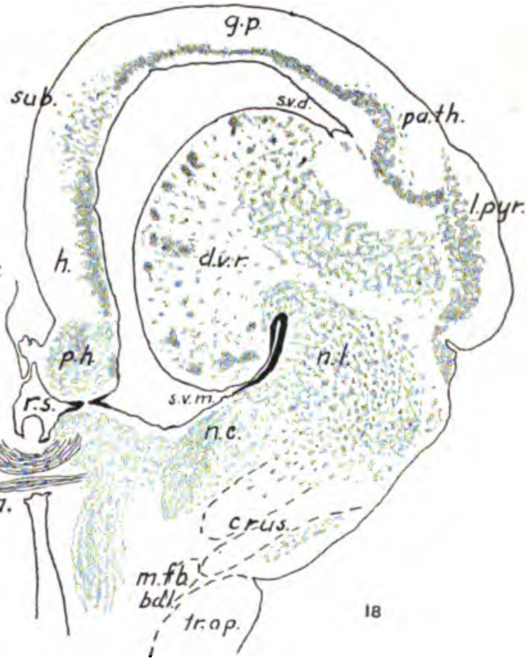
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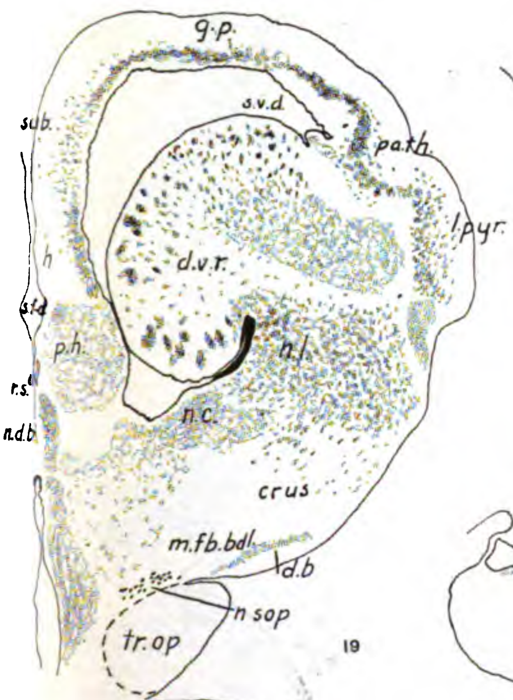
Fig. 19 Section in front of the commissures and behind the optic chiasma. The lentiform nucleus is large; the crus occupies the clear space below it and sends fascicles up through it (internal capsule). The nucleus of the diagonal band forms a thin cell layer between the fiber layer of that band and the medial forebrain bundle. The caudate is small. The upper end of the nucleus of the diagonal band appears here in the gyrus subcallosus adjacent to the hippocampal primordium and the recessus superior. Note the distinct large- and small-celled portions of the pyriform lobe. Also the general increase in thickness and more open formation of the cell layer of the dorsal pallium.

Fig. 20 Section through the optic chiasma. The ventricular ridge grows smaller and the definite pallial thickening begins. The hippocampus proper grows narrow. The hippocampal primordium is only apparently fused with the parolfactory nuclei, as explained in the previous paper. The diagonal band and its nucleus move to the angle between the basal and medial walls. The sulcus endorhinalis separates the small-celled from the large-celled part of the pyriform lobe. The lentiform nucleus is quite superficial in this and several following figures. The clear zones above and below the core-nucleus of the ventricular ridge and between that and the pyriform lobe are the spaces through which internal capsule fibers run to the ridge and the general pallium.

Fig. 21 Section at the level of the gyrus subcallosus. The changes noted in the last section have been carried farther. The lentiform nucleus shows rows of cells due to the arrangement of bundles of internal capsule. In this and figure 22 the diagonal band nucleus merges with the superficial layer of the anterior perforate space and tuberculum olfactorium.

Fig. 22 Section through the anterior end of the dorsal ventricular ridge. The core-nucleus is here divided into two areas by pressure from the pallial thickening. This thickening is the most conspicuous feature of the section. Note the development of a layer of peripheral cells in figures 20, 21 and 22. Compare figure 40. The caudate begins to enlarge and the lentiform to decrease.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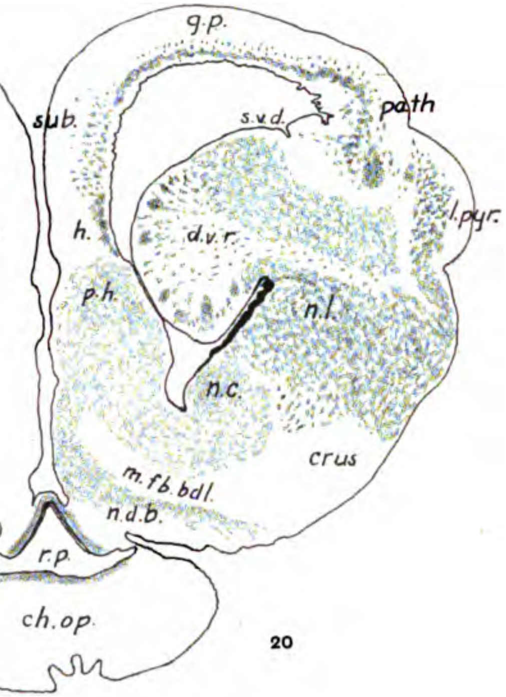
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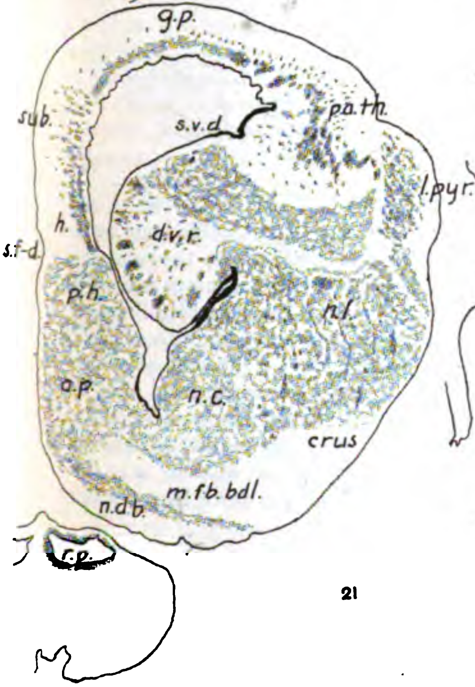
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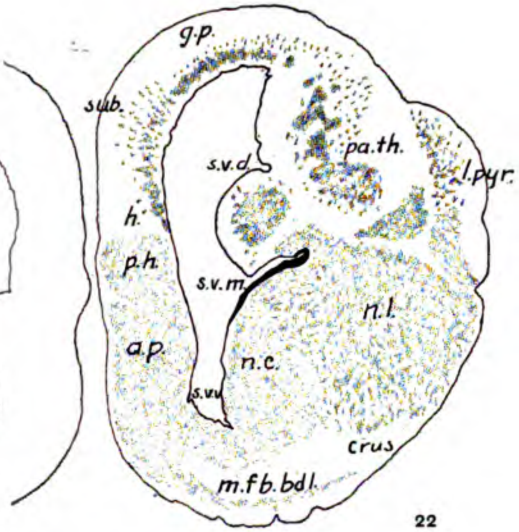
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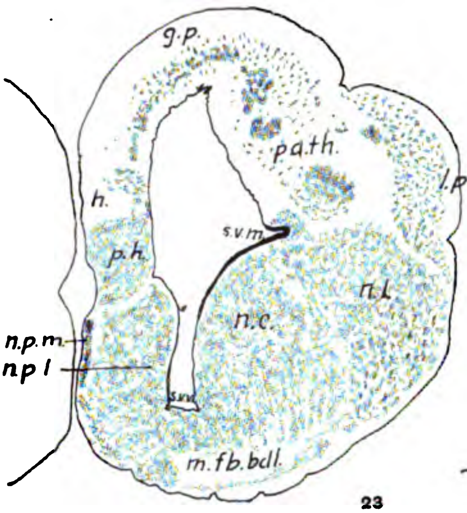

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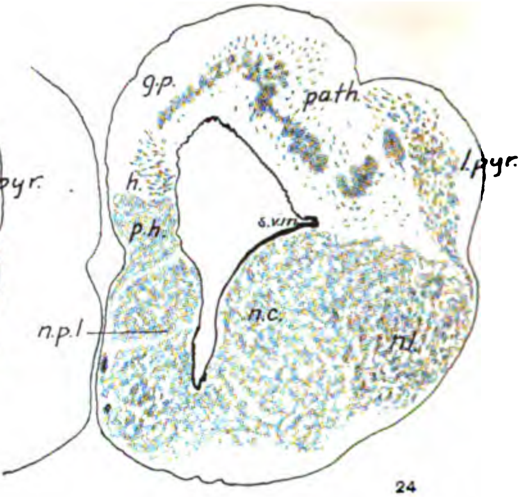

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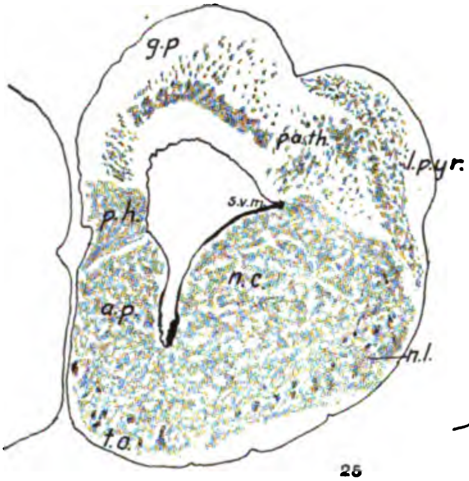

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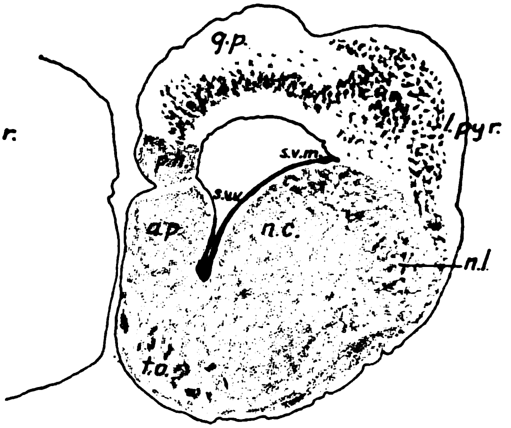

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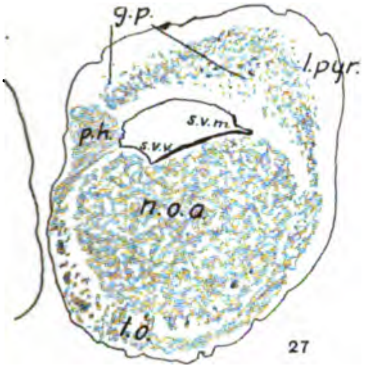
Fig. 23 Section through the pallial thickening in front of the ventricular ridge. In this and the next three figures the pyriform lobe is bulged out by the pallial thickening. Although the lentiform nucleus still meets the ventricle, the caudate is greatly enlarged. From here forward the hippocampus is scarcely if at all recognizable. The subiculum continues between the general pallium and the hippocampal primordium. The dividing line between primordium hippocampi and parolfactory area is again clear. In this section the ventral ventricular groove reaches its greatest depth and width. Forward from this the thickening of caudate, parolfactory nucleus and tuberculum nearly obliterate it.

Fig. 24 Section through the middle of the pallial thickening. Islands of Calleja begin to appear in the medial wall. The lentiform nucleus is pushed toward the surface by the caudate. For a detail of the pyriform lobe and part of the pallial thickening compare figure 40.

Fig. 25 Section through the caudal part of the tuberculum olfactorium and the head of the caudate. Note the high dorsal prominence caused by the pallial thickening. This scarcely shows satisfactorily in the figures of the whole brain or model. A part of the apparent thickness of the cell layer is due to the section being slightly oblique to the curvature of the dorsal pallium but the appearance of a distinct peripheral layer of cells is not due to this. Islands of Calleja are grouped in the medio-basal angle. Dark masses farther laterad are merely clumps of large cells in the lentiform nucleus.



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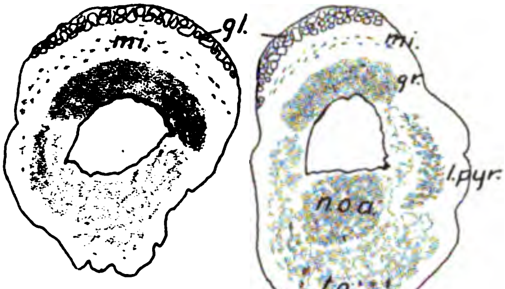

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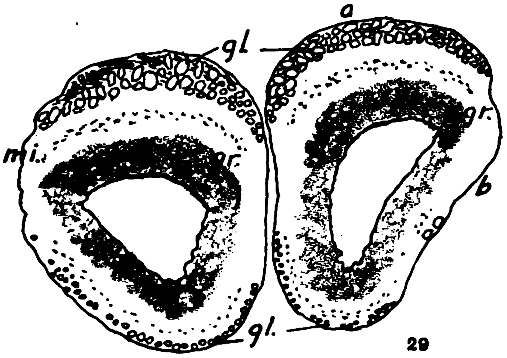

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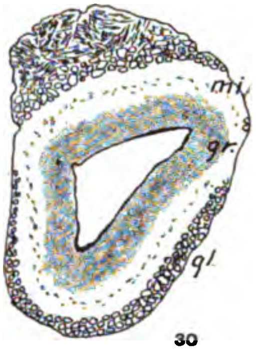

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Fig. 26 Section a short distance in front of the last. It shows the rostral end of the lentiform nucleus and the spreading of the small-celled part of the pyriform lobe over the striatum as the superficial layer of the tuberculum. The whole width of pallium in figs. 25, 26 has the characters of the thickening.

Fig. 27 Section through the rostral part of the tuberculum. The section passes just behind the peduncular constriction dorsally. Olfactory formation begins to appear 225 microns farther forward. The head of the caudate together with the deep or lateral parolfactory nucleus form one rounded mass covered externally by tuberculum. This is the nucleus olfactorius anterior of authors. Islands of Calleja are in the deep layer of the tuberculum. The tuberculum is continuous with the medial parolfactory nucleus and with the pyriform lobe. The pyriform lobe spreads medially in dorsal wall. General pallium is still present toward medial border and in some clusters of cells beneath pyriform lobe.

Fig. 28 Section through the olfactory peduncle. Both sides are shown, the left side being sectioned near the rostral end of the oblique peduncle, the right side near the caudal end of the peduncle. On the right side fully developed olfactory formation appears in the roof; in the lateral wall the pyriform lobe; below, the end of the nucleus olfactorius anterior and the tuberculum.

Fig. 29 Section through the caudal part of the olfactory bulb. Note the elliptical form of section on the right, the greater number of cells where olfactory glomeruli are present and the distribution of mitral cells with reference to the glomeruli. *a*, *b*, regions of this section which are drawn in figure 31.

Fig. 30 Section through the anterior part of the right bulb. Note the triangular form of section and the fact that the dorsal root of the olfactory nerve is just entering the bulb. The bulb is nearly surrounded by glomeruli and the mitral cell layer is continuous except for a short space on the lateral surface where glomeruli are absent.

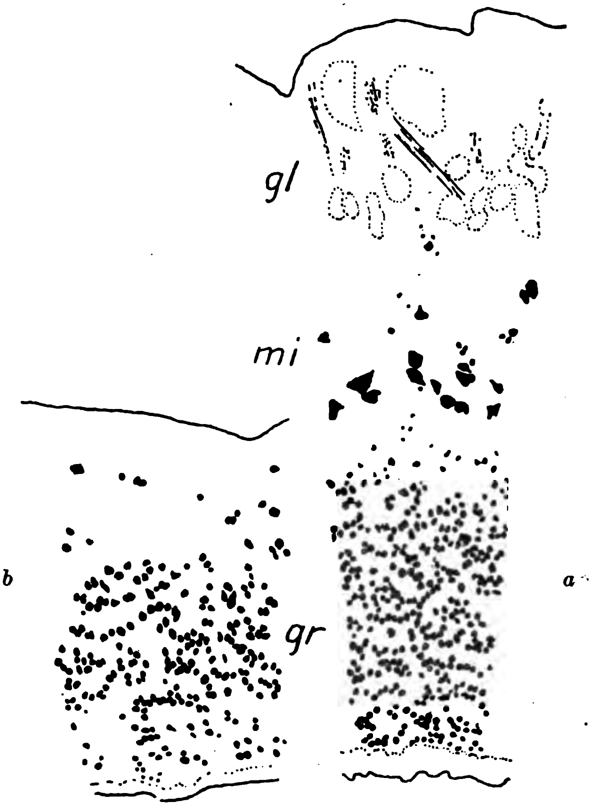
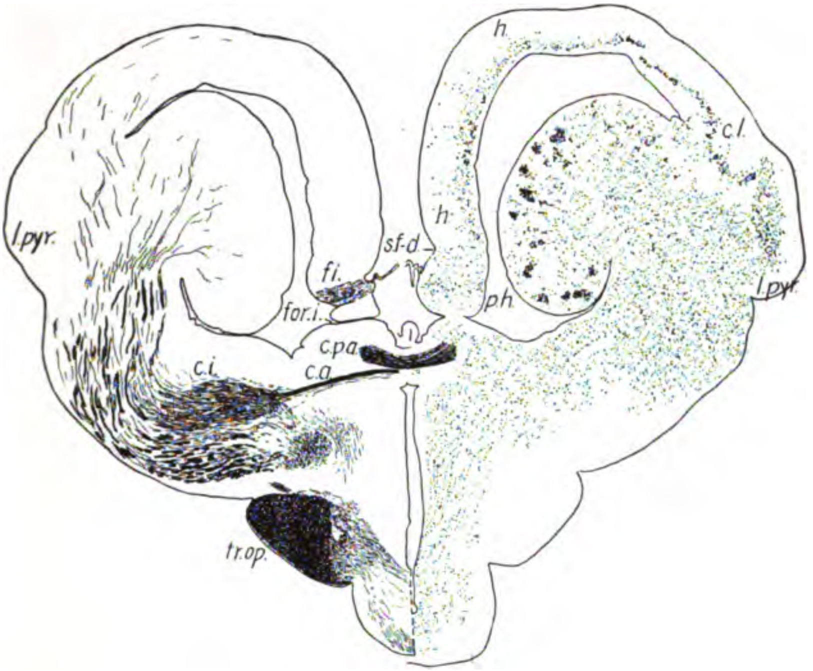


Fig. 31 Two portions from the section drawn in figure 29 to show the modification of the structure of the olfactory formation due to the presence of glomeruli. The part of the section drawn in *a* and *b* respectively is indicated by the same letter in figure 29. Where glomeruli are present the granule cell layer is considerably thicker and more dense and the layer of large mitral cells is added. Magnification 110 diameters.

Fig. 32 Transverse section at the level of the commissures. From Johnston '13b, fig. 17. The right side of the drawing was taken from a section adjacent to that from which figure 18 of the present paper was drawn. The left side was drawn from a Weigert series. The crus shows the two chief bundles described in the text. To what extent the fascicles of the two bundles intermingle as they are distributed to the pallium and the ventricular lobe it is impossible to say from sections of normal brains. The relation of the anterior commissure to the internal capsule is better shown in figure 48. Medial to the crus appear two other bundles. The darker one is the medial forebrain bundle, the lighter is the olfactory projection tract.

Fig. 33 A section similar to the last, taken at the level of the stria medullaris. From Johnston '13b, fig. 18. The two bundles of the crus are separated by a notch medially. The medial forebrain bundle is ventral to the olfactory projection tract and in contact with the postoptic decussation. The lower part of the fiber area beneath the letters *c.i.*, belongs to the olfactory projection tract. The stria medullaris is more superficial.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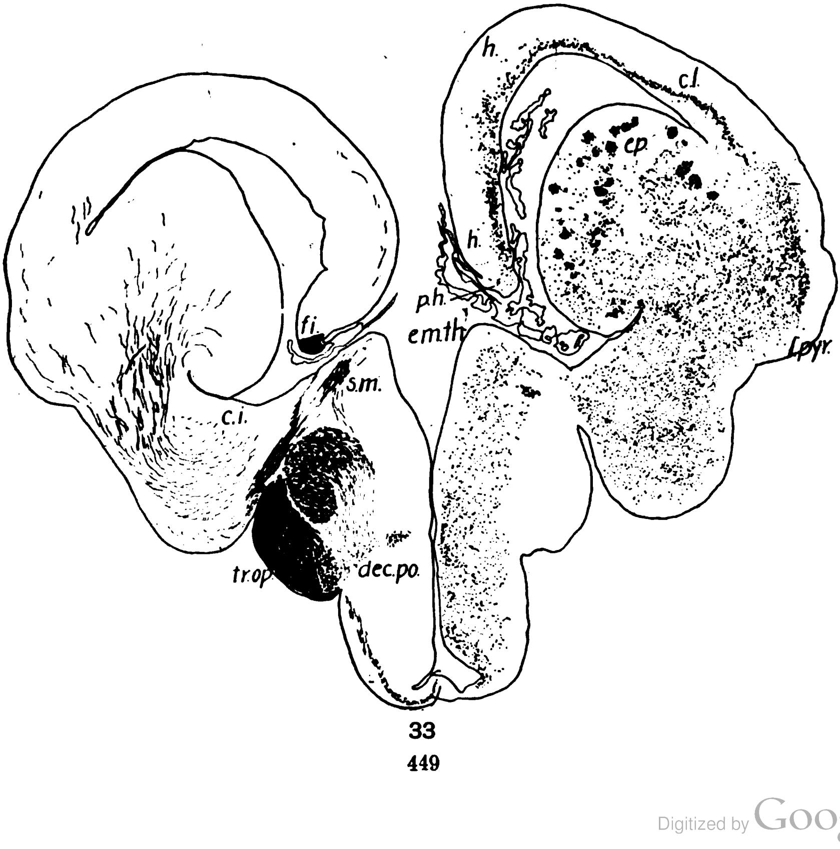
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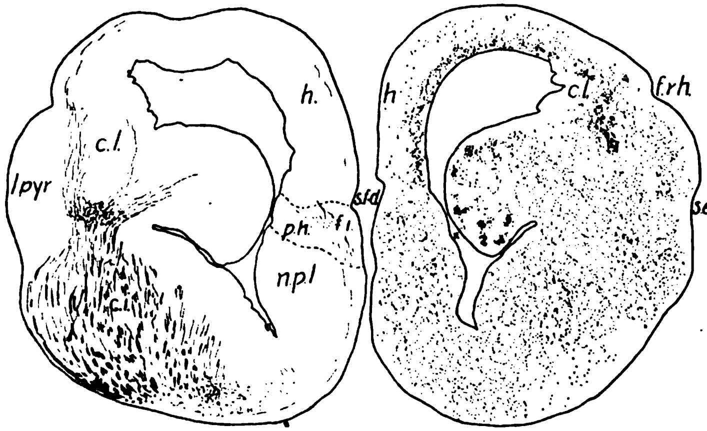

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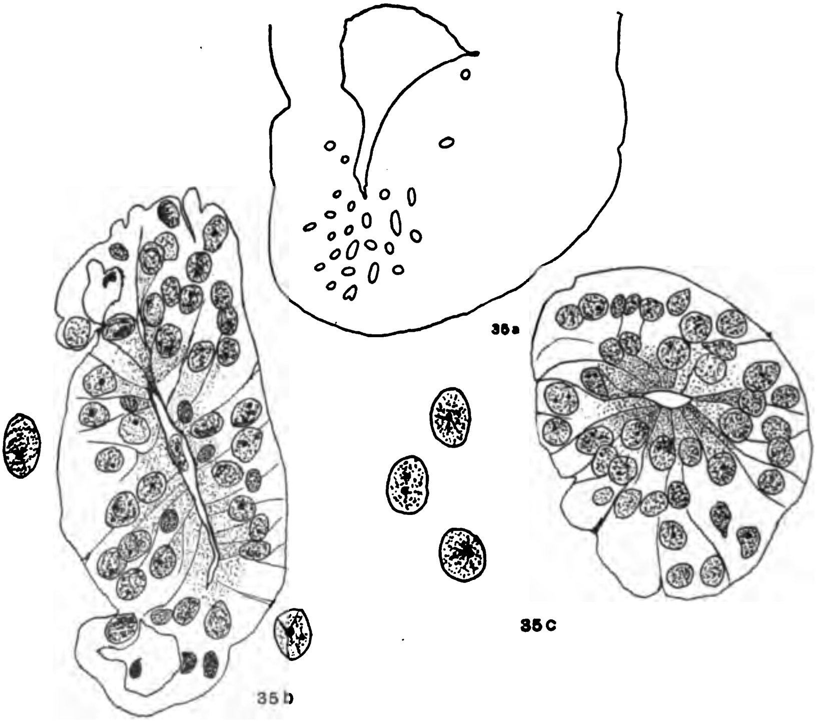

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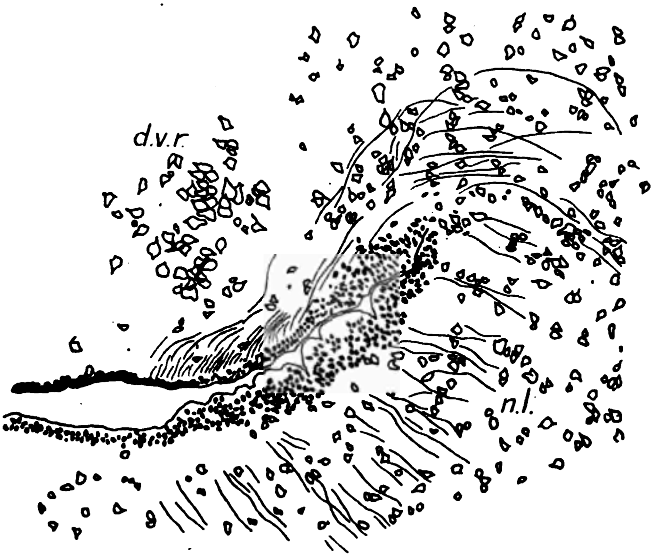

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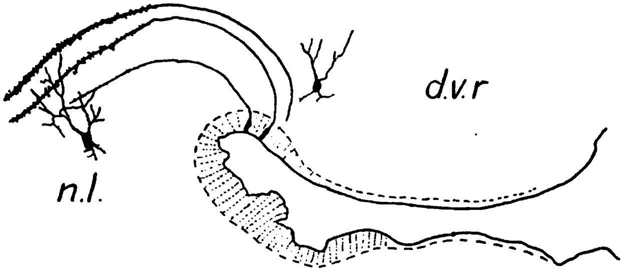
Fig. 34 A section similar to the last, taken near the rostral end of the ventricular ridge. The internal capsule fibers are related largely to the pallial thickening (*c.l.*). The medial part of the fiber bundles below belongs to the medial forebrain bundle. Its fibers are related only to the caudate and olfactory nuclei.

Fig. 35 Vesicular structures in the caudate nucleus. *a*, outline of a transverse section to indicate the position in which such vesicles are found. This is nearly at the same level as figure 25. Vesicles are found both rostral and caudal to this level. *b*, an oblique section of a vesicle the lumen of which contains a flattened nucleus of unknown character. Two blood corpuscles are seen. Vesicles often have capillaries closely connected with them. *c*, a transverse section of a vesicle with very sharply defined lumen. Note the granular contents of the inner ends of the cells. There are also faintly stained granules in the lumen. The isolated nuclei in *b* and *c* are nuclei of nerve cells lying near the vesicles. Magnification in *b* and *c*, 625 diameters.

Fig. 36 Section of the thickened ependyma surrounding the middle ventricular groove including a part of the lentiform nucleus. The nerve cells are drawn in outlines, the nuclei of the ependyma cells in black. The lines represent the peripheral processes of the ependyma cells. From the same section as figure 16. Magnification 112 diameters.



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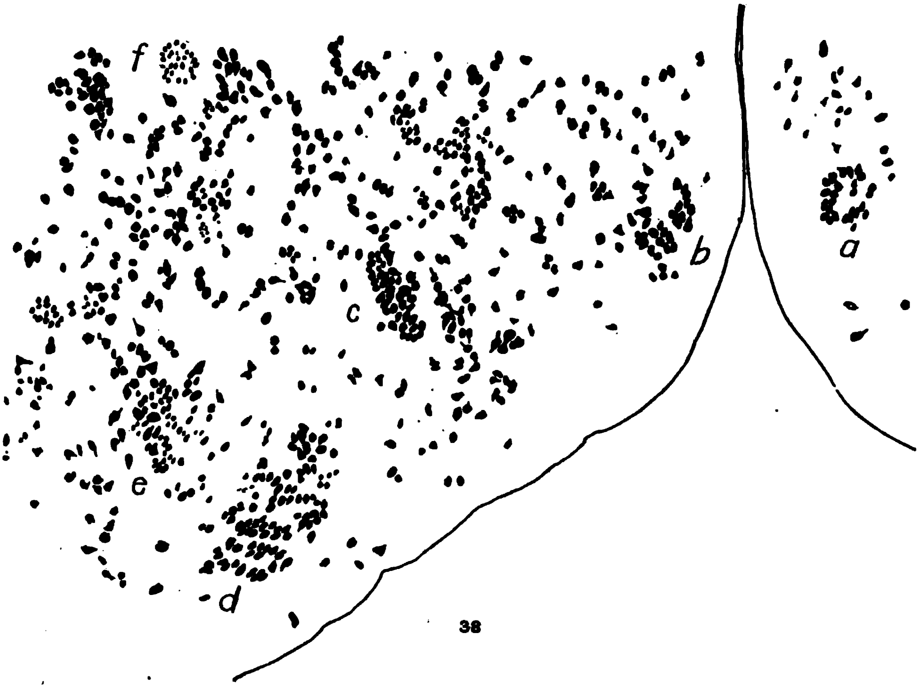

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Fig. 37 Same region as figure 36 but on the left side of the brain. A drawing from a Golgi section to show the ependyma cells in the dorsal lip of the middle ventricular groove, and their curved processes.

Fig. 38 Transverse section through the tuberculum olfactorium to show the grouping of cells in the islands of Calleja. From a section adjacent to that shown in figure 24. Magnification 110 diameters. The medio-basal angle of the left side and a small part of that of the right side are included. *a*, *b*, *c*, *d*, typical islands. *e*, an island in which larger and small cells are mingled. In some islands similar to this the small cells definitely show the vesicular arrangement with lumina. *f*, a vesicle similar to those shown in figure 35. Only the nuclei are drawn.

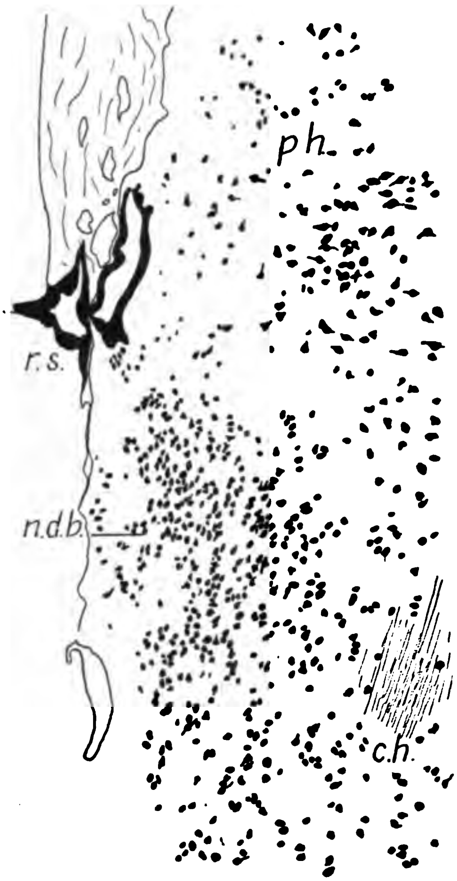


Fig. 39 Transverse section through the upper end of the gyrus subcallosus, showing the dense nucleus of the diagonal band close to the recessus superior. From the same section as figure 19. The position of the hippocampal commissure shows that this nucleus pushes up near the medial surface above the level of the commissure. This is shown in the model, but the ventral border of the hippocampal primordium in front of the commissure curves dorsally still more than is shown in the model (fig. 6). Note how much smaller these cells are than those of the adjacent hippocampal primordium. Indeed these are among the smallest cells in the forebrain. Magnification 110 diameters.

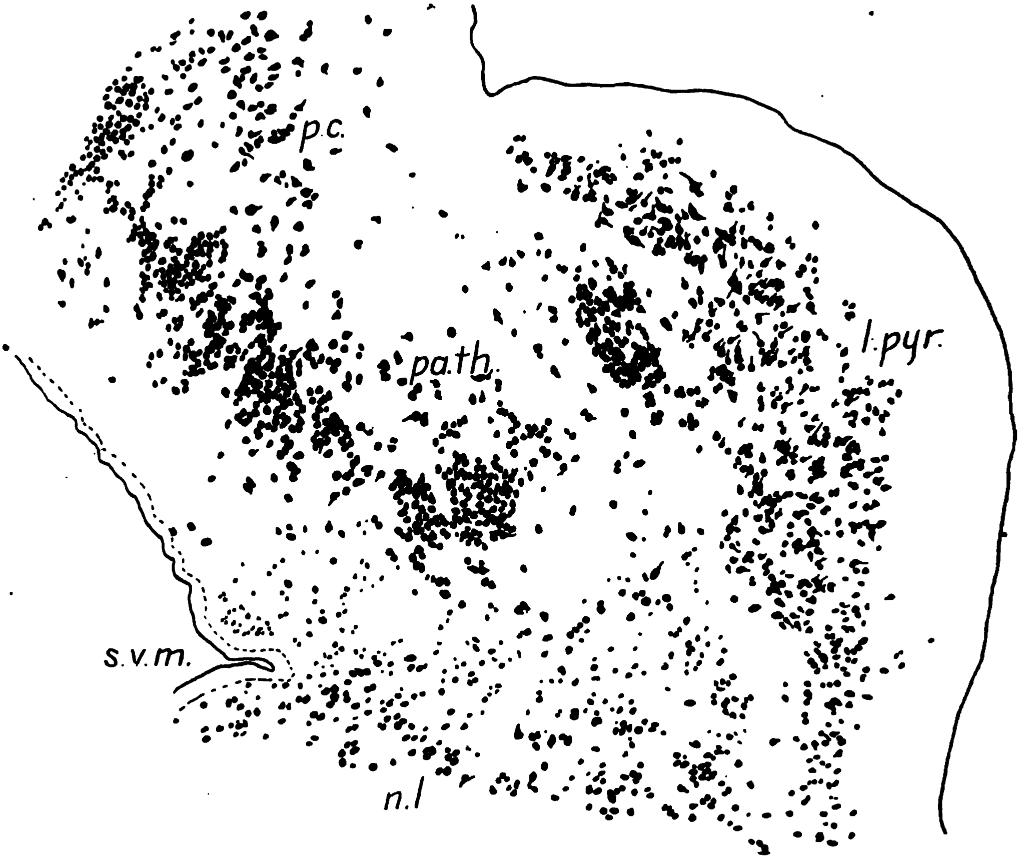


Fig. 40 Transverse section through the pyriform lobe and the pallial thickening to show the form and grouping of the cells. From the same section as figure 24. Magnification 70 diameters. In the upper part of the figure enough of the pallium is drawn to show the importance of the peripheral layer of cells. The deeper cells are smaller and so closely crowded that their processes seldom show at this magnification. The thick dendrites of the larger cells in the pyriform lobe, however, are clearly seen. It is evident that the large cell clusters belong to the pallium and not to the pyriform lobe.

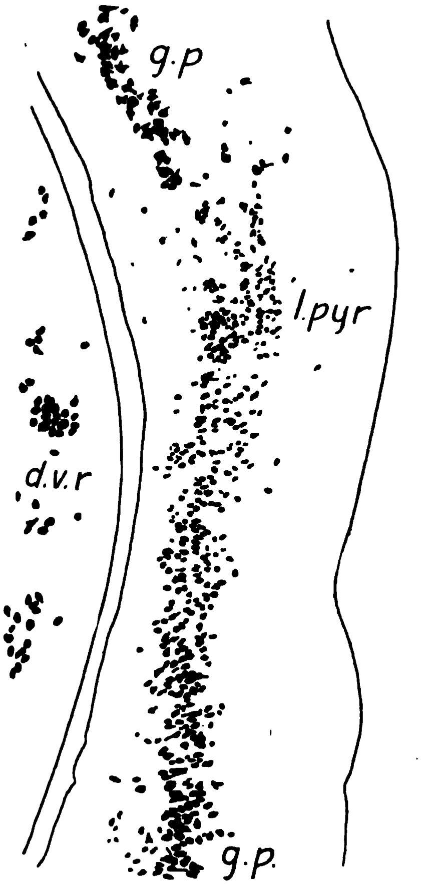
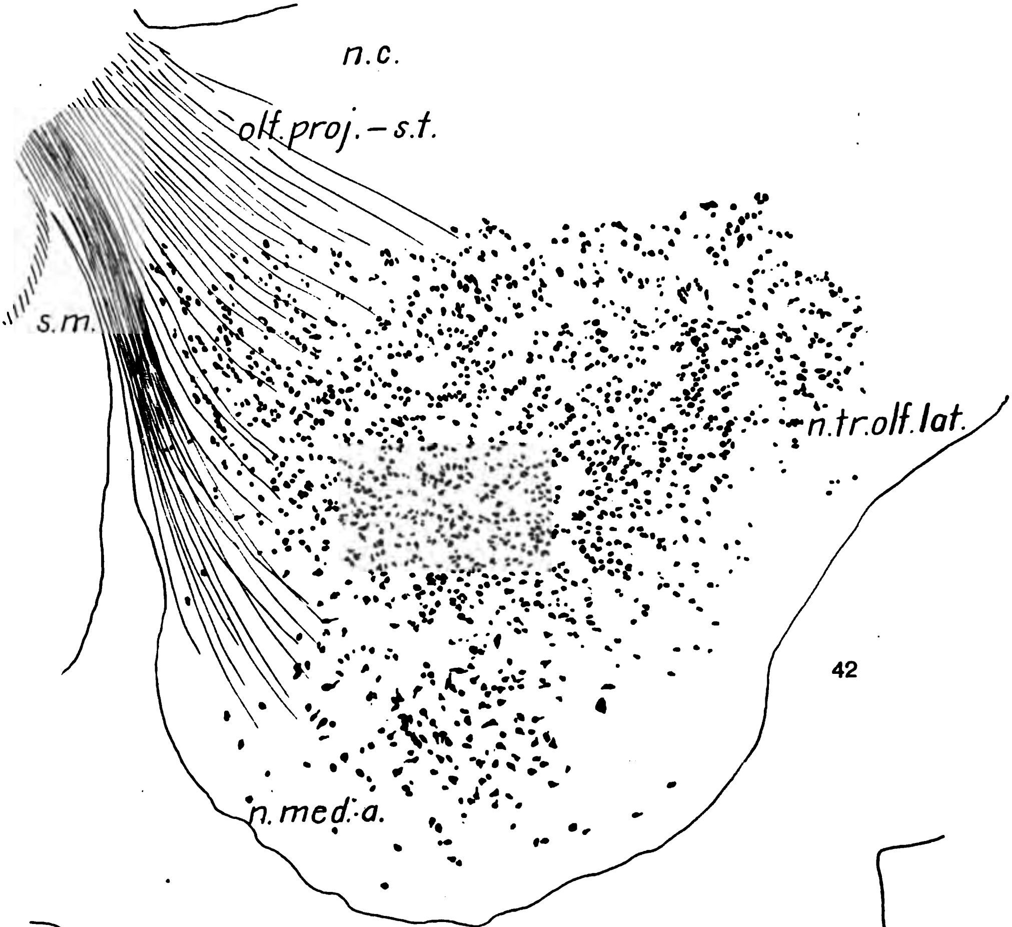


Fig. 41 Transverse section of the caudal part of the pyriform lobe. From the same section as figure 13. Magnification 70 diameters. In the upper part of the figure is seen the sharp dividing line between pyriform lobe and general pallium. No well-marked line of division is seen below.

Figs. 42 to 45 Four transverse sections through the region of the medial nucleus of the amygdaloid complex. From the same sections as 16, 15, 14, 13 respectively. Magnification 70 diameters.



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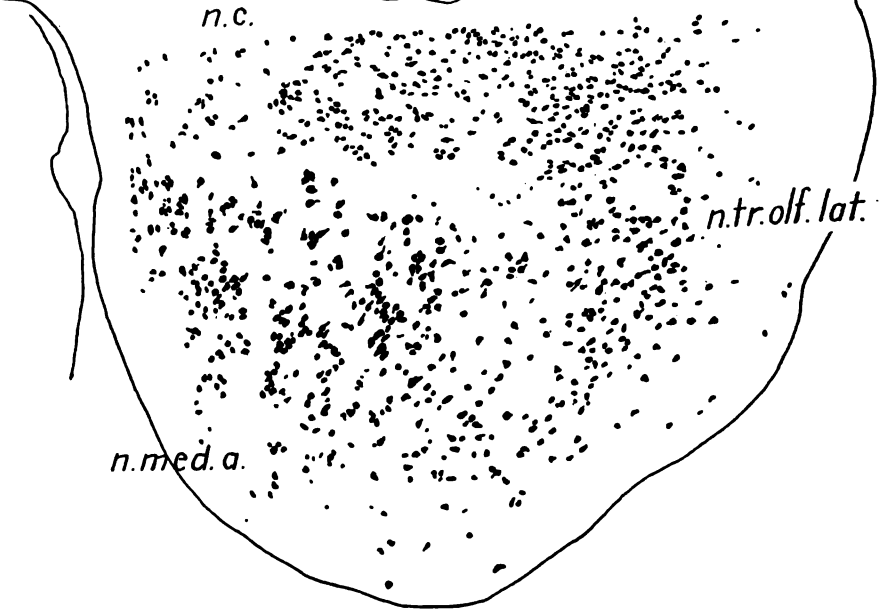

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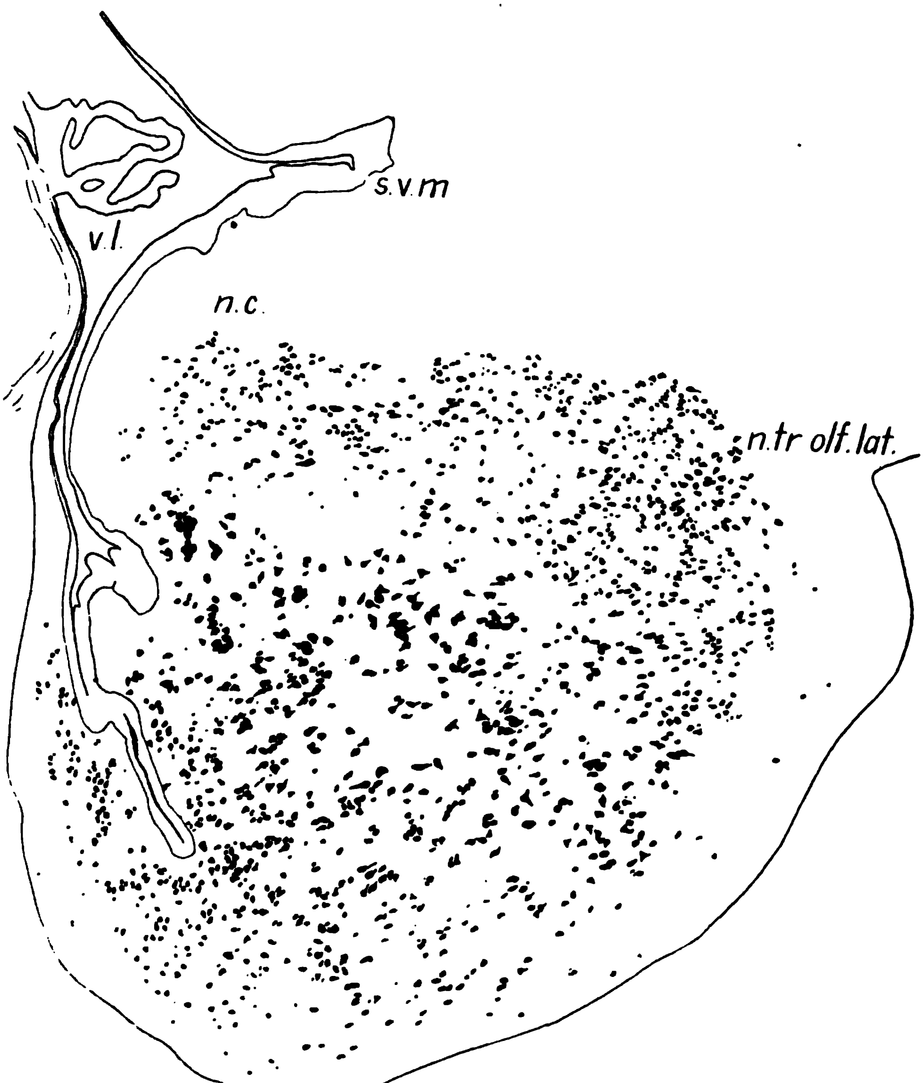

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Fig. 42 In this is seen the common mass of small cells formed by the caudate and the nucleus of the lateral olfactory tract. Below it is a mass of neuropile in which appear a considerable number of larger cells belonging to the medial nucleus. The stria medullaris enters into this neuropile while the olfactory projection tract and stria terminalis in part are related to the small-celled nucleus (compare fig. 49).

Fig. 43 Only a little of the small-celled mass is shown. The medial nucleus is larger and more dense and has shifted toward the medial wall.

Fig. 44 The medial nucleus is still larger and surrounds the temporal horn of the ventricle, the rostral end of which is cut by the section. Immediately caudal to this the medial wall of the ventricle is occupied by hippocampal formation.

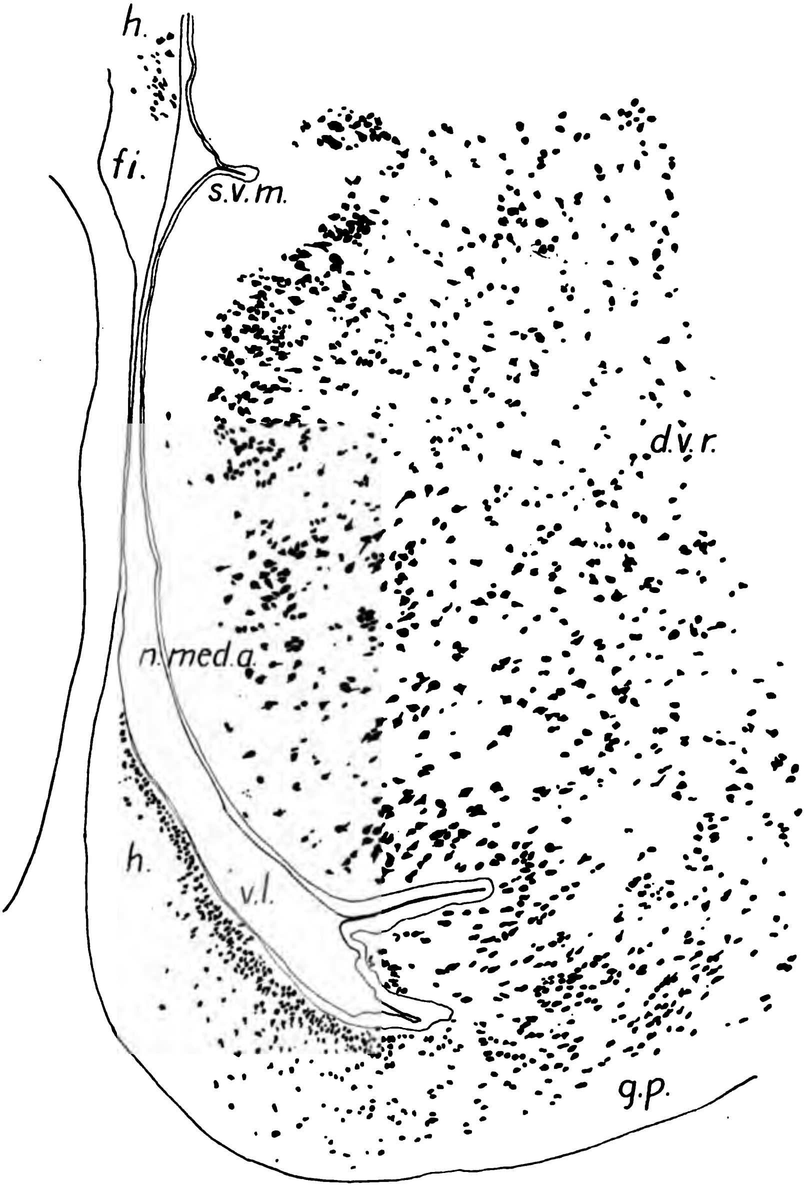


Fig. 45 In this section the medial nucleus occupies the ventricular surface of the basal lobe of the great ventricular ridge. Ventrally this mass of cells merges with the general pallium. The dense lamina in the medial wall belongs wholly or in part to the hippocampal formation. The small ridge in the lower angle of the ventricle lodges part of the stria terminalis bundle which spreads over the ventricular surface of the medial nucleus.

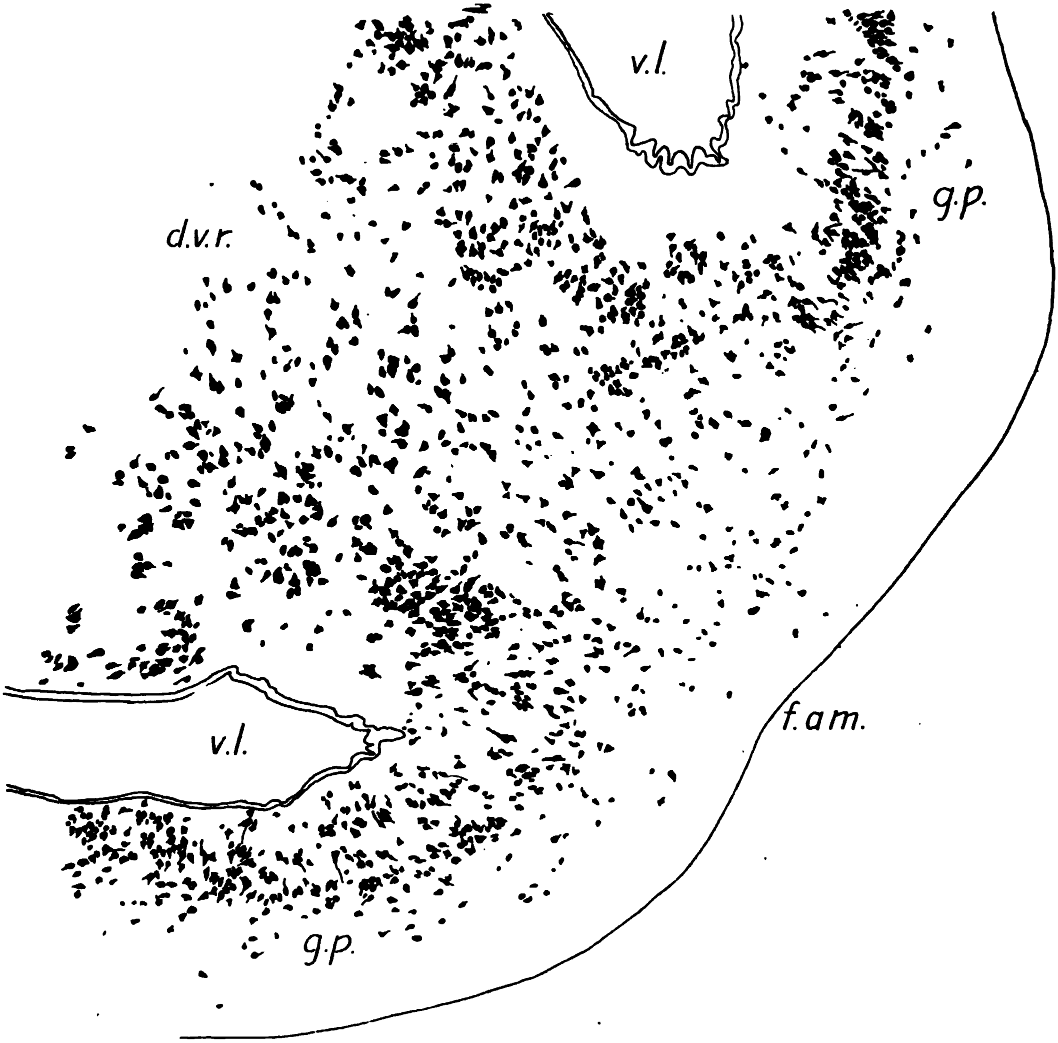
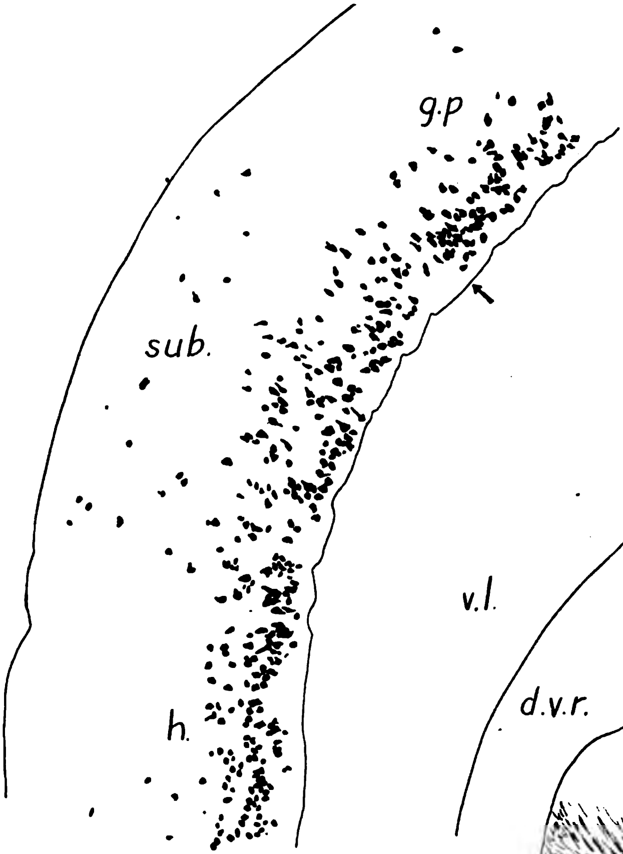


Fig. 46 Transverse section through the base of the ventricular ridge near its caudal end. From the same section as figure 11. Magnification 70 diameters. The relations are described in the text. The figure shows typical pallial walls laterally and ventrally which appear to be involuted to form the ventricular ridge.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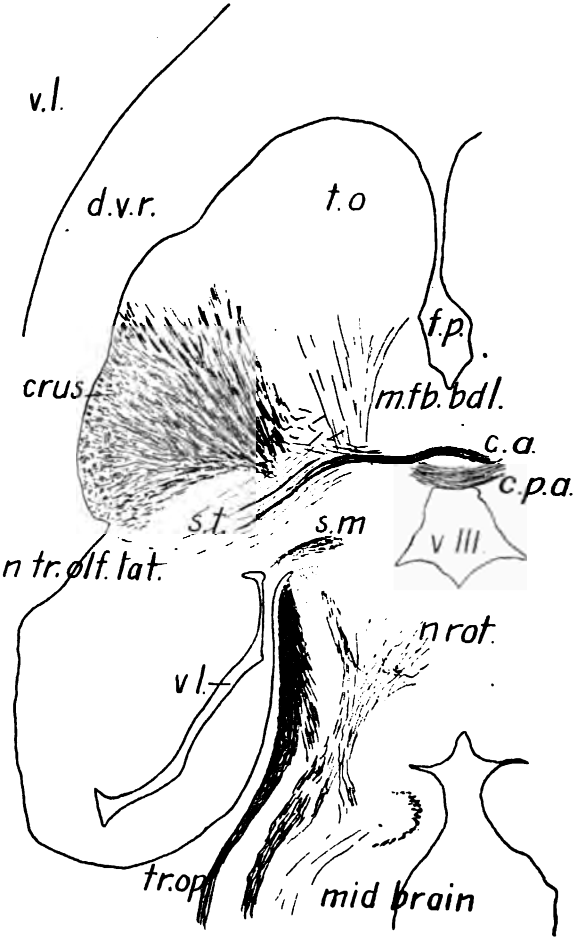
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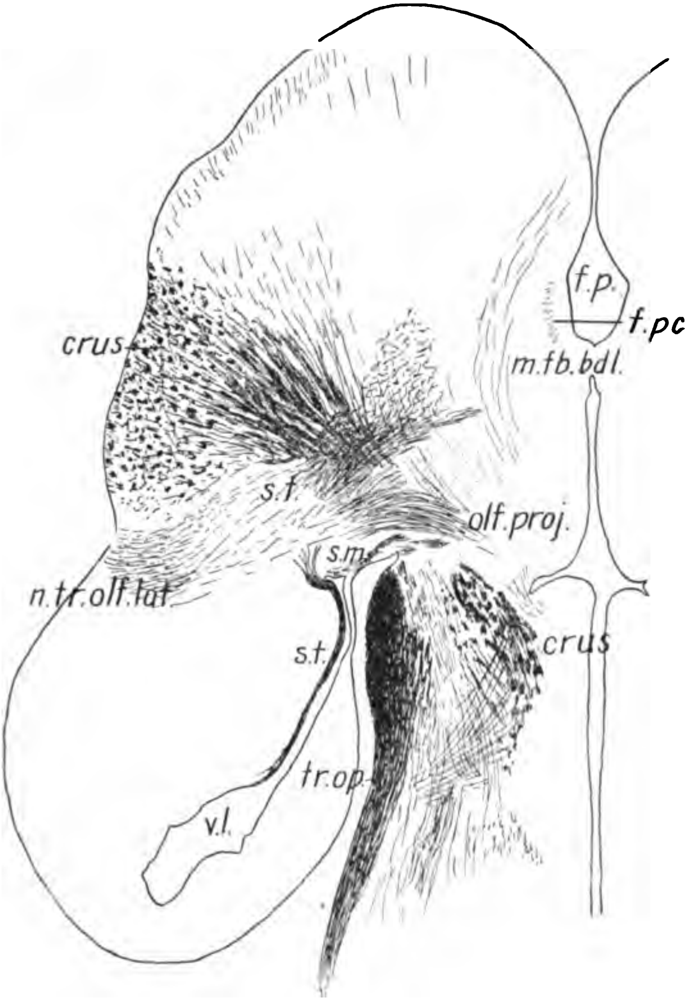

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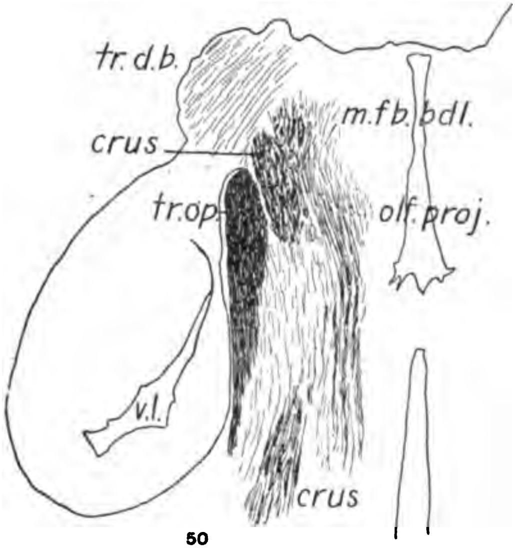
Fig. 47 A drawing of the subiculum region from the same section as figure 17. Magnification 70 diameters. The figure shows very inadequately the relatively dense layer of the hippocampus below, the more open arrangement of the cells in the subiculum and the abrupt medial border of the general pallium above. This border is marked by the arrow.

Fig. 48 Horizontal section from a Weigert series to show the anterior commissure and stria terminalis. The section is taken just at the level of the most dorsal part of the stria terminalis where it curves over the internal capsule. At this point the anterior commissure meets the stria (compare fig. 32) and continues with it into the small-celled nucleus of the amygdaloid complex.

Fig. 49 Horizontal section somewhat ventral to the level of that drawn in figure 48. This is from another series. The section passes through the lower or ventral part of the stria terminalis where it lies close upon and a little intermingled with the internal capsule. The section is drawn to show that the stria terminalis and the olfactory projection tract are divisions of a common broad bundle arising from the small-celled part of the amygdaloid complex. The bundle which enters the stria terminalis from the basal lobe of the ventricular ridge is drawn although it is almost wholly non-medullated. It can not be traced farther forward in these sections.



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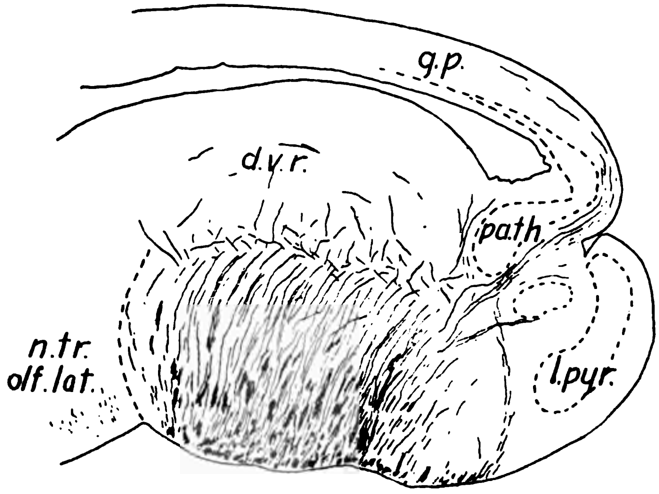

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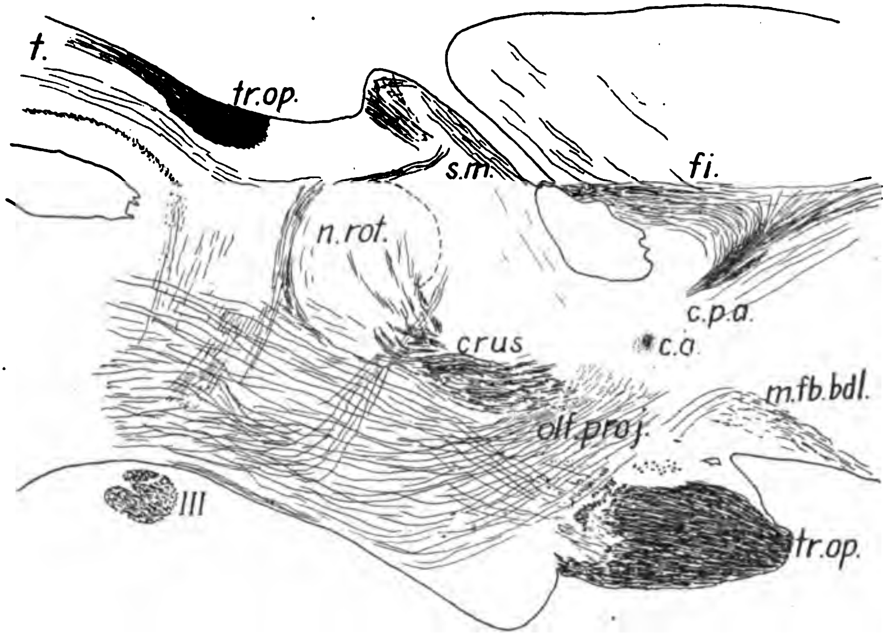
Fig. 50 Horizontal section through the temporal pole and thalamus. Same series as figure 49. This section shows the fiber bundle of the diagonal band where it crosses the basal surface of the crus and medial forebrain bundle.

Fig. 51 Parasagittal section close to the right lateral surface. Weigert stain. The cell masses of the pyriform lobe and pallial thickening are outlined in broken lines; also the line of division between the striatum and the amygdaloid complex. The figure shows especially the fibers of the internal capsule entering the pallial thickening.

Fig. 52 Section from the same series as the last. Near the median plane. The section shows the hippocampal commissure rising in the hippocampal primordium to join the fimbria system. There are shown also the relations of the sensory bundle of the crus, the olfactory projection tract and the medial forebrain bundle.



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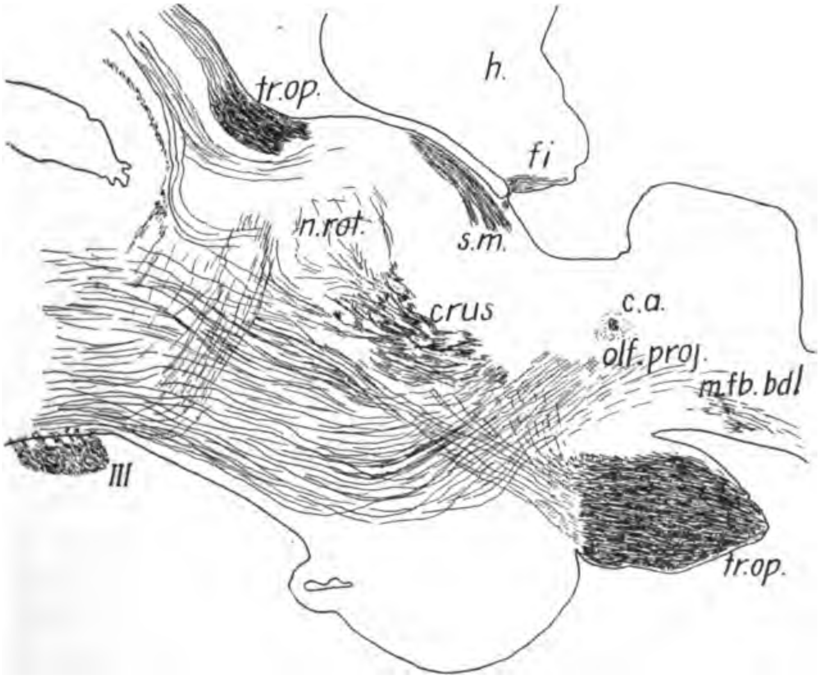


Fig. 53 A section from the same series as the last. It is taken from the left side of the brain. Owing to the oblique plane of the sections this is near the median plane at the level of the third nerve, but farther from the middle in the forebrain than in figure 52.

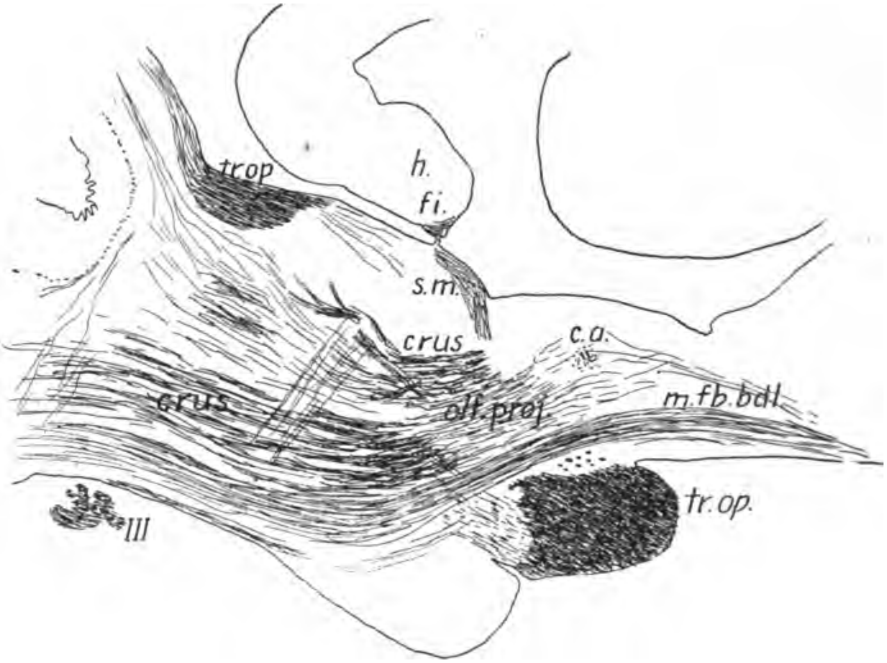
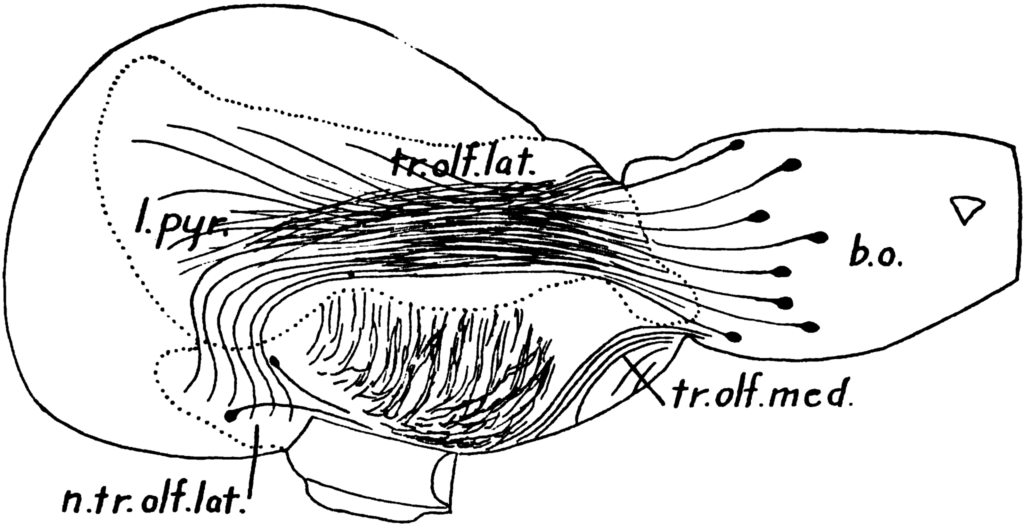


Fig. 54 A section from the same series, farther laterad than the last. This section shows a part of the sensory and the motor bundle of the crus, the olfactory projection tract and the median forebrain bundle. It is especially interesting to note from these sections that both the olfactory projection tract and the median forebrain bundle run almost wholly to the tegmental region or farther caudad, whereas it has been supposed that they both end for the most part in the hypothalamus.

Fig. 55 A diagram of the lateral olfactory tract together with a part of the medial olfactory tract and of the bundle of the diagonal band. Outline taken from figure 5. The figure shows the bundle which bends down behind the striatal area to end in the nucleus of the lateral olfactory tract in the amygdaloid eminence. At *a* is seen the bundle which comes from the medial wall of the olfactory bulb, crosses over the dorsal surface in the peduncular groove and joins the lateral tract. The whole course of the diagonal band can not be seen in this lateral view.

Fig. 56 A diagram of the olfactory tract in ventral view. Outline taken from figure 8. The medial and lateral bundles of the olfactory tract are separated by the striatal area containing the fibers of the crus. A bundle of the lateral tract curves around lateral and caudal to this area to reach the nucleus of the lateral olfactory tract, while a bundle of the medial tract passes along the ventral border of the striatal area to reach the same nucleus. From this nucleus, then, fibers enter the stria medullaris and the bundle of the diagonal band. The latter go to the hippocampus by way of the precommissural fornix system.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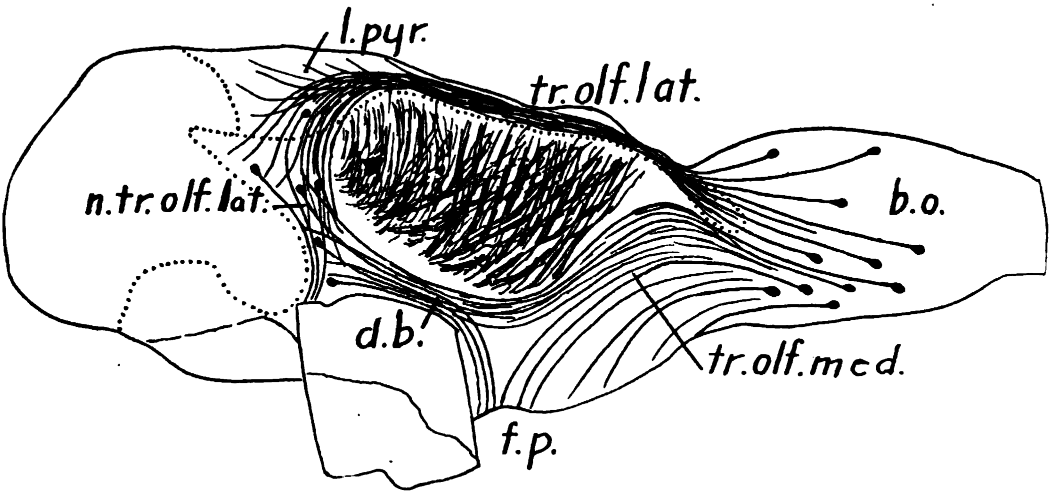
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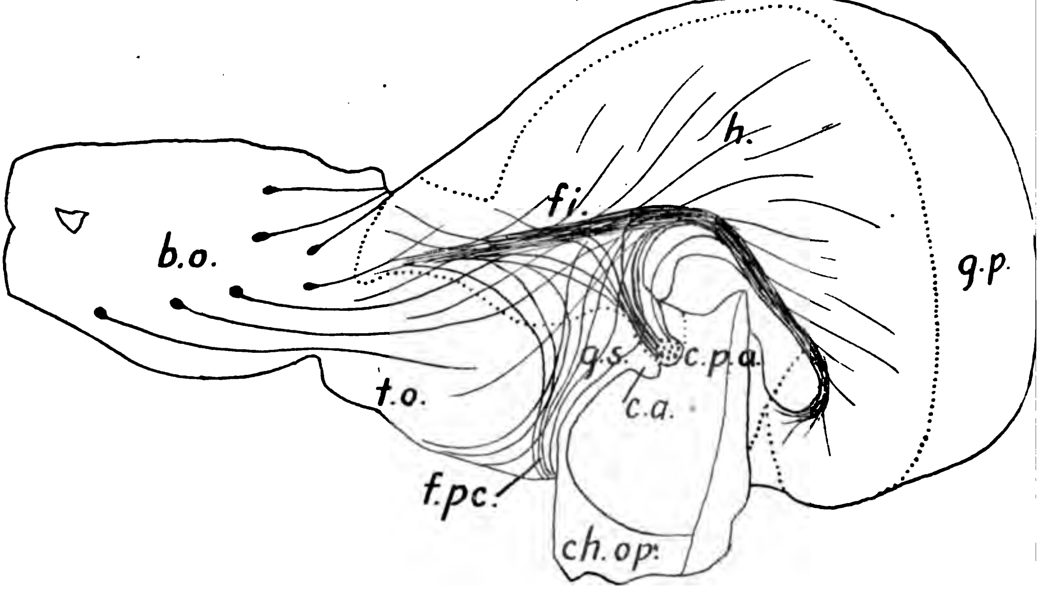

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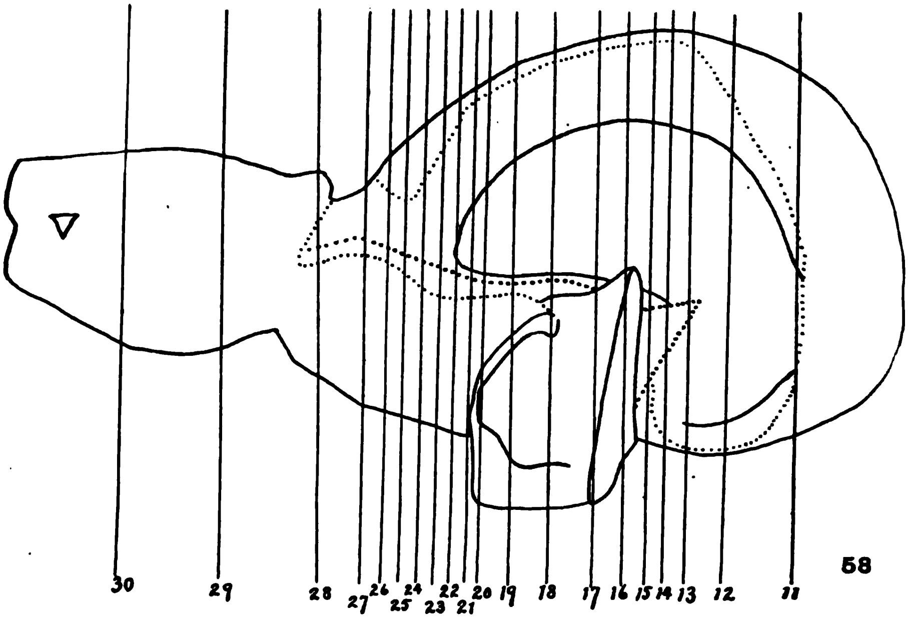

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Fig. 57 A diagram of tracts in the medial wall. Outline from figure 6. This shows especially how the fimbria system is made up of several components: olfactory tract fibers, precommissural fibers from the diagonal band, hippocampal commissure, and fornix (not drawn).

Fig. 58 Sketch of the right hemisphere after figure 10 with lines to show the position of the sections drawn in figures 11 to 30.

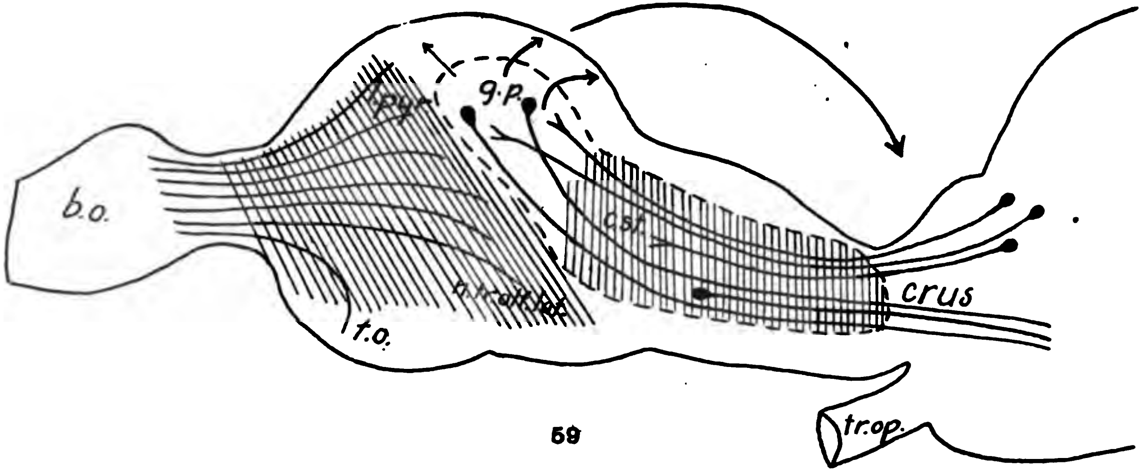

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Fig. 59 Schema of the selachian forebrain viewed from the left side. The outline corresponds to that of the forebrain in Scymnus or Heptanchus. The lateral olfactory area is shaded with oblique lines, the corpus striatum with vertical lines. The expansion of the somatic centers in the roof as general pallium is indicated by arrows and the direction of the chief expansion of the hemisphere is indicated by a large arrow. The equivalent of the pyriform lobe and the nucleus of the lateral olfactory tract lie rostral to the corpus striatum. The expansion of the pallium has been accompanied by a great elongation of this olfactory area.

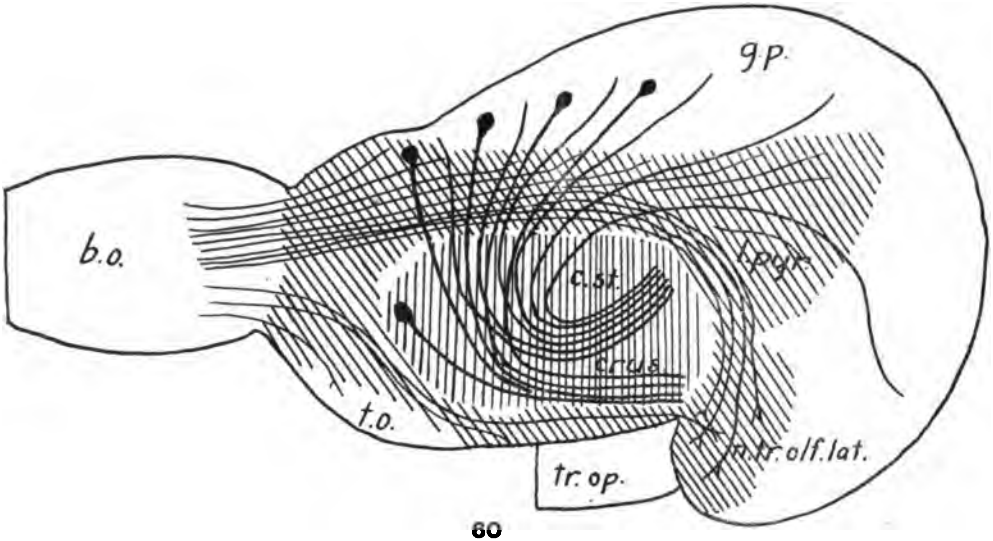

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Fig. 60 Schema of the forebrain of the turtle viewed from the left side, for comparison with figure 59. The olfactory area has been stretched over and beyond the corpus striatum but does not now form a covering of the striatum. The present condition in the turtle is such as would have been produced if the striatum had been covered by an olfactory layer and then the cells of that layer had withdrawn to the borders of the striatum so as to form a ring of olfactory centers around it.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 1, 1915

31. *On the implantation and placentation in the Sciuroid rodents (lantern).*
THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

In 1902 and 1903 the writer published descriptions of the implantation of the ovum in Spermophilus. In this work attention was called to a method of implantation and a series of structural changes preceding the formation of the true placenta which were unlike those of any other previously described mammal, and at the same time were the first account of the implantation in any of the Sciuroidae. These observations were confirmed on the European Spermophilus by Rejsek. In 1905 Müller found similar conditions in the European red squirrel, Sciurus. In 1910 the writer described the early stages of Cynomys at the International Anatomical Congress in Brussels. Since 1902 the writer has been engaged in collecting early stages of various genera of American Sciuroid rodents to determine if the peculiar conditions found in Spermophilus (or 'Citellus' as the taxonomists have since decided upon as the proper generic name) were characteristic of this large group of rodents. The collection of very early stages of wild rodents which breed for the most part but once a year, and whose genera are widely separated over the United States, is an extremely tedious and very expensive undertaking, but sufficient material has been secured to date of the following genera of the Sciuroidae to determine that the general method of implantation and placentation as previously described for Citellus (Spermophilus) hold true for the larger division. The genera studied include Citellus, 3 species, Ammospermophilus, Tamias, Cynomys, and Sciurus. The writer is preparing a more complete description and illustration of the early developmental conditions characteristic of these Sciruoidae than was possible before with the quite limited material. The greater variety of material now available enables one to point out the interesting slight divergences among the several genera.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 1, 1915

32. *On the relationship of the endocardium to entoderm in Citellus.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

In studying the early development of the sciuroid rodent Citellus the writer noted the following described conditions which may be of interest to investigators working on that yet unsolved problem of the origin of the vascular system.

With the folding over and fusion of the entodermal walls of the foregut to form the pharynx region, there is to be noted a dorso-lateral angle on either side formed by the dorsal wall on either side of the chorda and the lateral wall of the pharynx and a somewhat less prominent ventro-lateral angle or groove, which will be designated in this paper as the cardiac sulcus.

Each cardiac sulcus is a groove or furrow in the free surface of the entoderm which follows the course of the lateral hearts. It begins in the lateral wall of the mid gut region and extends forwards to enter the closed pharynx region at the ventro-lateral angle and then continues along the ventral wall of the pharnyx converging to unite with the opposite sulcus in the mid ventral line just above the point of fusion of the lateral hearts.

The entoderm in the line of the cardiac sulcus is considerably thicker than that on either side. This thickening, however, is not uniform; it is more pronounced in certain areas than others. There is thus produced a corresponding elevation or ridge of the entoderm in the direction of the lateral heart. This thickened entoderm constitutes the walls of the sulcus. The groove, while easily recognizable throughout its course, varies in its shape; in places it is narrow and deep, in other places it becomes widened out and quite shallow. In embryos of this stage of development, the fold of splanchnic mesoderm which will form the myocardium does not completely envelope the endothelial tube of the lateral heart, the interval being completed by the entoderm of the foregut. It is this portion of the entoderm that forms the cardiac sulcus. The above described sulcus is not peculiar to Citellus but is figured by many investigators, as Kölliker in the rabbit, Fleischman in the cat, Bonnet in the dog. It is a transitory structure but is probably to be found in all mammals at the proper stage of development. While this region has been figured, almost no reference has been made to it as far as I am at present familiar with the literature.

In a number of series of Citellus I have found interesting examples of an intimate relationship between the entoderm of the cardiac sulcus and the endocardium of the lateral heart as shown by the reconstructions and drawings that illustrate this paper.

In the Citellus embryo here modelled, the primary implantation attachment (previously described by the writer) is just separating while the trophoblastic attachment for the allantoic placenta is beginning at the mesometrial portion of the uterine cavity; the amnion is not yet quite complete; the foregut is closed, the ectoderm of the oral plate is fused with the entoderm but not broken through; the pharynx does not yet show the evagination of the pouches; the endocardium of the lateral heart is beginning to fuse at the anterior end; the two dorsal aortae are well outlined, the first aortic arch is not yet completed. In an embryo at this stage the endocardium of the lateral heart on either side, in the region between the junctions of foregut and midgut and the point of beginning union of the two lateral hearts, shows an intimate relationship to the thickened entoderm of the cardiac sulcus. The endocardial tube is free and separate from the myocardial fold of splanchnic mesoderm in the sections, the contour of the tube is either oval or pear-shaped with a portion of the endothelial wall extended out as a thin fold or strand of cells toward the sulcus. Examining the series section by section it will be seen that while in each there is the extension of the fold or strand of cells toward the sulcus, in certain sections there is a short interval and in others very close contact; in certain sections there is distinct continuity with the entoderm of the sulcus walls. This intimate relationship is lost in the region of the fusion of the lateral heart.

Reprinted from THE ANATOMICAL RECORD, Vol. 8, No. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

39a. The passage of the ovum through the uterine epithelium in Geomys bursarius, with demonstration of wax reconstructions. THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

Within the order of Rodentia there exists a greater variety in the implantation of ovum and formation of decidual cavity than in any of the other main divisions of the Mammalia. The rage of variation extends from those forms, like the rabbit, in which the whole of the uterine lumen is utilized, to that of mouse and rat, where only a restricted portion of the uterine cavity is transformed into a decidual cavity. Then follows those peculiar forms, as Citellus (spermophilus), in which the writer in 1902 and 1903 described for the first time a rodent in which the trophoblastic layer of cells at close of segmentation caused the destruction of a small area of the uterine epithelium at the antimesometrial portion of uterine cavity, followed by an outgrowth of trophoblastic cells into the mucosa to form a nutrient organ, followed by the atrophy and disappearance of this organ upon the completion of the allantoic placenta at the mesometrial portion of the uterus; the whole of the uterine cavity being utilized for a decidual cavity, as in the rabbit. And lastly are found those forms in which, as in man, the ovum passes entirely through the uterine epithelium and a new decidual cavity is formed in the mucosa and independent of the uterine lumen. The first rodent of this type to be described was the guinea pig, so beautifully worked out by Graf Spee. In Geomys we find a second rodent of this type, but with the following important points of difference from the guinea-pig which are shown in these reconstructions. In the guinea-pig the ovum passes through the uterine epithelium at close of segmentation, and while it is very small in volume, the small perforation is quickly closed and the uterine cavity completely separated by epithelium from the new decidual cavity. In Geomys, on the contrary, the ovum perforates the uterine epithelium in the blastula stage, and when of so large a diameter that the edges of the large rounded perforation of the uterine epithelium cannot grow together as in the guinea-pig, or be filled with a fibrin plug as in man, this opening persists during the entire preplacental period. The epithelium at the lip of perforation is somewhat everted and gives a point of attachment to a zone of the trophoblastic layer of the blastocyst. The dorsal portion of the trophoblastic layer extends across the opening, and by this zonal attachment to the epithelial lip completely shuts off the uterine lumen from the new decidual cavity. The cells of the mucosa are broken down and the decidual cavity is rapidly enlarged. The blastocyst sinks down into this cavity but·continues to be suspended by the above described zonal attachment to the lip of the epithelial perforation. This zone will ultimately form the outer margin of the allantoic placenta. A detailed description of the unique preplacental development in Geomys with plates will be published in the near future.

Reprinted from THE ANATOMICAL RECORD, VOL. 8, NO. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

39b. An improved electric microscope lamp, with demonstration. THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

This lamp was devised by the writer to meet the demand for a small compact and portable lamp for individual staff or student use in the Minnesota Institute of Anatomy, and has proved to be so satisfactory that it is here demonstrated for the benefit of the members of the Association. The lamp consists of a small vulcanite base fitted with a silvered reflector and a socket into which can be screwed as desired either a 2, 4 or 6 candle-power Mazda lamp with miniature base; a metal cap supported by 3 rods which fit into the base shuts off all side light and gives the necessary ventilation. In the top of this cap is an opening the same size as the base of the Abbe condensor. A device on the top of the metal cap holds in place over this opening the ordinary blue or ground glass plates commonly used with the Abbe condensor, thus giving at all times monochromatic light of uniform intensity. The base is fitted with flexible electric fixture wire terminating in a small plug which fits into a socket in the table top. The entire lamp is small, about two inches in diameter and in height, so that it readily fits in under the Abbe condensor when the mirror is pushed to one side. When not in use, the lamp can be put away in the student's locker with the rest of his outfit. A stock automobile Mazda bulb of standard make of 6 volts, 5 watts, alternating current is used in this lamp. It is the smallest available, the least expensive, and will withstand rough usage. In fitting up the laboratory tables a step down transformer is connected with the feed wire. This reduces the current used from 110 volts to 6 volts. These transformers are small, inexpensive, and can be had of any capacity desired, are used in the trade in sign lighting apparatus. The wires leading from transformer are run underneath the tables and connected to the sockets which are set into table top at any desired point. A detailed description of this apparatus with illustrations will appear in the near future.

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

41. *Studies on the syrinx of Gallus domesticus.* J. A. Myers, Institute of Anatomy, University of Minnesota, Minneapolis.

The results of this work may be summarized as follows:

Structure. (1) The syrinx of the domestic chicken belongs to the tracheo-bronchialis type, and is quite simple when compared with the voice organ of song birds. (2) No intrinsic muscles are present in the syrinx of Gallus domesticus. The extrinsic paired sterno-trachealis with its caudal prolongations constitute the entire musculature of the syrinx. (3) The rigid skeleton is very highly modified. The first four tracheal rings are imperfectly fused to form the tympanum. The four intermediate syringeal cartilages are continuous ventrally with the ventral pyramid of the pessulus, while dorsally they end unattached. The first bronchial half-rings are large and in adults are attached and fused at both ends of the pessulus. The pessulus is the largest of all skeletal parts and lies dorso-ventrally at the junction of the bronchi in a plane transverse to the long axis of the trachea. The tracheal rings, the pessulus, and the ventral ends of the first half-rings become ossified, while all other skeletal parts remain cartilaginous. (4) The external tympanic membranes appear between the fourth intermediate syringeal cartilages and the first half-rings while the internal tympanic membranes extend from the caudal borders of the pessulus to the bronchidesmus and represent merely a modified part of the medial bronchial walls. (5) The syrinx is lined with stratified ciliated columnar epithelium containing numerous simple alveolar glands. Upon approaching the tympanic membranes this columnar epithelium is transformed into a stratified squamous epithelium which becomes a single layer of flattened cells over the membranes proper. (6) The tympanum is attached to the remainder of the syrinx only by elastic membranes.

Development. (1) The first indication of the respiratory system was observed in a 68 hour embryo in which the laryngeo-tracheal groove and the bronchi were represented. At first the trachea is much shorter than the bronchi, but with the development of the neck, it becomes, after the 140 hour stage, relatively much longer than the bronchi. The walls of the trachea and the bronchi are at first composed only of epithelium which contains two or three rows of nuclei. (2) The mesenchymal condensation common to the entire epithelial tube first becomes markedly prominent at the tracheal bifurcation in an embryo of 152 hours. (3) The anlagen of the first bronchial half-rings appear in a 176 hour embryo, those of the fourth intermediate syringeal cartilages appear 12 hours later. The anlagen of the third intermediate syringeal cartilages and the anlage of the pessulus are present at 200 hours. (4) Distinct cartilage cells were first observed in the first bronchial half-rings. (5) The first four tracheal rings have not united to form the tympanum at hatching, nor have the other skeletal elements taken the shape of those found in the adult. No bone is present at the time of hatching. (6) Ciliated cells are present in stages beyond 248 hours but were not observed in the region of the future tympanic membranes. (7) Mucous cells were first observed in 332 hour embryos and only in later stages were they found arranged in the form of simple alveolar glands. (8) Muscular tissue is differentiated in the 176 hour stage. Muscle fibers showing faint cross striations appear at 296 hours. At 452 hours the muscles are well developed. (9) At the time of hatching the tympanic membranes are thick. They are covered, however, as in the adult, with a single layer of epithelial cells.

Function. (1) That the syrinx is the true voice organ of the chicken is evident from the following deductions: First, structurally it is the only part of the respiratory tract capable of producing sound; Second, when the trachea is divided and the cephalic portion tightly tied off, the chicken is still able to crow; Third, after division of the trachea, voice can be reproduced artificially by forcing air into the air sacs. (2) The upper larynx serves only to modulate the voice. (3) The sterno-tracheal muscles by their contraction shorten the trachea and modify pitch. They also draw the tympanum cephalad, thus indirectly varying the tenseness of the tympanic membranes. (4) The air sacs are necessary in voice production, for voice could not be reproduced artificially after puncturing the cervical sacs.

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48. The development of the pancreas in selachians. Richard E. Scammon, Institute of Anatomy, University of Minnesota.

The pancreas of selachians is remarkable in that it arises from a single diverticulum which has generally been described as dorsal in position. It is, however, very difficult to determine the exact position of this anlage by the customary means of reconstruction. For this purpose, therefore, I have applied the method of graphic reconstruction devised by Weber. This method, which seems to me a most valuable device for studying the early stages of the larger glands, has apparently been employed only by its originator some ten years ago in an extensive study of the earliest stages of development of the liver and pancreas in Amniotes. The details of this procedure can hardly be given in the space allotted here. They may be found in Weber's original work (Arch. D'Anat. microsc., T. 5, '03), and will be again presented in this paper in its final form. In general terms, it is a modification of the graphic method of reconstruction in which the varying thickness of the epithelium of the reconstructed archenteron is represented in corresponding varying shades of color so that one may study the various areas of epithelial thickening in much the same way that one interprets the elevations of land in a topographic map.

A reconstruction made in this manner of the midgut of an Acanthias embryo 5.2 mm. in length, which is of a stage well preceding any indication of the pancreas as an outpouching, shows in the future pancreatic region of thickened epithelium which includes not only the dorsal zone of the gut but extends well ventrally. This pancreatic area is connected with a large thickening ventral and anterior to it which constitutes the liver anlage. These two thickenings, the pancreatic and hepatic, constitute, with the thickened epithelium connecting them, a ring which passes obliquely completely around the archenteron. The existence of such a pancreatic-hepatic ring was postulated long ago by Brachet and has been demonstrated to a great extent in Amniotes by Weber.

Reconstructions by Weber's method of the midgut regions of embryos of Acanthias 7.5, 9 and 10 mm. in length, show a gradual breaking up of this ventral hepatic segment. Between the two the epithelium remains somewhat thickened and in this occurs a particularly thickened patch which occupied the same position as does the anlage of the ventral pancreas in the higher vertebrates. This thickened plate never produces an outpouching and disappears in older embryos. While the dorsal outpouching of the pancreas is a single one, there is in Acanthias at least, an early indication of division into right and left lobes. This division is not very distinct and can hardly be demonstrated without Weberian and plastic reconstructions. In Acanthias the left lobe lies anterior to the right and is the smaller. It forms in embryos from 15 to 35 mm. in length a distinct antero-ventral mass which later is to a considerable extent incorporated in the descending limb of the gland. In such other selachians as I have studied this lobe lies posterior to the right one. This is probably because the left lobe is not differentiated until the gland begins to separate from the gut and in all selachians except Acanthias separation takes place antero-posteriorly. I do not think that the bilobed dorsal pancreas is to be regarded as a primitive condition. Rather it is produced by the clockwise rotation of the gut and the formation of the spiral valve. Bilobed dorsal pancreas anlagen are found in mammals and in selachians but have not been clearly demonstrated elsewhere unless one accepts the observations of von Küppfer the value of which has been rendered doubtful by the work of Piper and Nicolas. It seems then that the bilobed form of dorsal pancreas is limited to those forms in which the clockwise rotation of the gut (common to some extent to all vertebrates) takes place at an early period and that this form of pancreas is due to that rotation.

A more complete statement of the development of the selachian pancreas will be published in the near future.

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

45. *The technique of Weber's method of reconstruction.* Richard E. Scammon, Institute of Anatomy, University of Minnesota. (Lantern slides.)

This method as used by its author was applied mainly to surfaces which were almost flat. Its extension to the study of rounded surfaces requires that a method of curvature elimination be adopted. In dealing with the surfaces of cylinders or cones this is easily done by correcting for vertical curvature alone, but where segments of spherical surfaces are involved corrections for horizontal curvature are also necessary. Vertical and horizontal corrections can be made so that the finished plat will give an approximately true representation of both the area and shape of a given outline upon a curved surface.

The method of making a finished reconstruction from the reconstruction plat has been considerably simplified by the introduction of color by means of 'Herring' papers and by building up the final reconstruction in strata.

The entire process involves the following steps: (a) Correcting section drawings for vertical curvature; (b) Scaling drawings for thickness; (c) Laying out reconstruction plat with corrections for vertical and horizontal curvature; (d) Plotting gauge lines; (e) Building up the finished reconstruction from the plat.

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ON WEBER'S METHOD OF RECONSTRUCTION AND ITS APPLICATION TO CURVED SURFACES

RICHARD E. SCAMMON
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FIVE FIGURES

In embryological work it is sometimes a matter of importance to determine with accuracy areas of epithelial thickening which are not demonstrated satisfactorily by the ordinary methods of graphic or plastic reconstruction. Placodal thickenings of the skin ectoderm, thickened zones in the neural tube, and the thickened areas found in the early archenteron are examples of structures which are not well demonstrated by these customary methods. A method of reconstruction which brings out graphically the extent and comparative thickness of such areas was devised some years ago by A. Weber, and was used by him with much success in the study of the very early development of the great glands of the digestive tract. Weber first published an account of his method in 1902,[1] and again a shorter summary in connection with the final report on his work in the following year.[2] Apparently the method has not been employed elsewhere. I have found it of such interest in the study of the earlier stages of the pancreas and liver that it has seemed desirable to present it here with some modifications which may be found useful, particularly in its application to curved surfaces.

Weber's method is based upon that of graphic reconstruction from transverse sections. An outline (from either lateral or dorsal view, as desired) of the organ to be reconstructed is plotted out on transverse section lines in the customary manner. Instead, however, of completing the reconstruction by indicating the *contour* of the surface thus outlined (as is commonly done), the *thickness* of the wall which forms that surface is measured in the transverse sections, and the variations in this thickness are plotted out upon the reconstruction. The reconstruction will then bear a number of lines which mark the boundaries between areas of epithelium of different thicknesses. To finish the reconstruction, the areas thus outlined are filled in with different shades of a single color, the darker shades being used for the thicker areas of the epithelial wall. The finished reconstruction then will exhibit the outline of the structure and the variation in the thickness of the part of its wall which is shown in this particular view. It will give no conception of the surface modeling of this wall aside from what can be determined from the outline alone.

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[1] Une méthode de reconstruction graphique d'épaisseurs et quelques-unes de ses applications à l'embryologie. Bibliog. Anat., T. 11.

[2] L'origine des glandes annexes de l'intestine moyen chez les vertébrés. Arch. d'Anat. Micr., T. 10.

The picture obtained is much the same as would be secured were it possible to remove the wall of the structure, render it translucent and, magnifying it greatly, hold it before a bright light. The thicker portions of the wall would then appear to the observer as darker areas, as they do in the reconstruction.

Figure 1 is a graphic reconstruction of the left side of the archenteron of an embryo of Torpedo ocellata, 4.0 mm. in length (No. 765 of the Harvard Embryological Collection). The portion of the gut lying on either side of the anterior intestinal portal and included between the lines *A* and *B* has been reconstructed by Weber's method and is shown in figure 4. The latter figure shows by means of its coloring a broad band of thickened epithelium extending dorso-ventrally across the gut at the level of the anterior intestinal portal. The lower and thickest part of this band represents the anlage of the gall bladder and liver, while the upper part includes the future pancreatic region. A thickened spur extends backward from this zone, and marks off the line along which the intestine will eventually separate from the yolk-stalk ventral to it.

The method of preparation of such reconstructions can best be explained in detail by following an example of the process.

For this purpose I will use the reconstruction shown in figure 4 which has just been described.

In preparing this reconstruction, drawings were made of each section of the portion of the gut involved, although fairly satisfactory results can be secured with drawings of every other section. These drawings should be made at a high magnification, preferably over 300 diameters. As the sections are drawn, they show of course the curvatures of the contour of the archenteron wall. It is desirable to eliminate these curvatures in the reconstruction and to present the gut wall as an approximately flat plane. If this is not done, areas which project sharply from the general plane of the archenteron will be represented in the reconstruction as much narrower than they actually are in the specimen or, if small, may be lost entirely. To eliminate these curvatures, it is necessary to divide the outer margin of each section into a number of short cords or segments, each of which will be comparatively straight, and to lay off segments of an equal length on the transverse section lines of the reconstruction. This can be done with a pair of small screw compasses. For work at a magnification of 300 diameters and over,

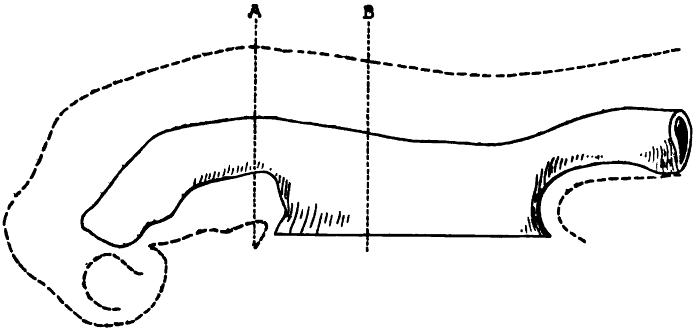

Fig. 1 A graphic reconstruction (lateral view) of a portion of the archenteron of an embryo of Torpedo ocellata 4.0 mm. long (H.E.C. 765). × 30. The outline of the embryo is represented in broken line. The archenteron is drawn in solid line. The portion of the archenteron lying between the dotted vertical lines *A* and *B* is shown in a Weber's reconstruction, at higher magnification, in figure 4.

segments of 1 cm. are small enough to eliminate most of the error from curvature. Figure 2 is of a section (No. 104) of the reconstruction under discussion. The short lines on the inner side of its margin represent the boundaries of these centimeter segments.

The thickness of the epithelium forming the gut wall is now to be measured in each drawing. For this purpose a unit of measurement must first be determined. Working with drawings made at a magnification of 300, it has been found that the 1.5 mm. is the smallest unit practicable for such a scale. By using this unit one is able to measure variations of less than 5 micra in the thickness of the gut wall, and errors of projection and drawing would probably render more refined measurements of little value. A scale of 1.5 mm. units is laid out upon a stiff card, or better, a piece of transparent celluloid. This scale or gauge is then passed over each section, care being taken to keep its graduated margin at right angles to the *axis* of each segment of the drawing and its zero point at the inner margin of the epithelium. This process is begun at the dorsal median line on each section and is carried laterally or ventrally from that point. At the first place where the thickness of the epithelium is found to correspond to a graduation point on the scale, a fine line is drawn out to the side of the section and the thickness noted at its end. The scale is carried along the section until a point is reached where the thickness of the epithelium corresponds with the next graduation (either above or below the former one) on the scale. Again a line is drawn out from the section at this point and the thickness noted. This process is continued until the entire side of the section has been gauged and marked off into segments in which the variation in thickness is not over 1.5 mm. on the drawing or 5 micra in the corresponding section. An example of a section thus gauged is shown in figure 2. As shown by the gauge lines, the epithelium immediately below the dorsal median line is between 30 and 25 micra (6.5 mm. at × 300) in thickness. The thickness falls to 25 micra at the point indicated. This is followed by a broad zone which is less than 25, but over 20 micra thick. Ventral to this zone, the epithelium increases to a thickness of between 35 and 40 micra, and then again decreases to less than 10 micra as it approaches the blastoderm. The arrows seen in the figure point towards the thinner edge of the segment and are used to avoid confusion in mapping out the gauge lines on the reconstruction at a later time. It is important that in gauging the section drawing the scale be held at right angles to the long axis of that particular part of the wall which is being measured rather than at a similar angle to either the inner or outer surface of the epithelial strip. This holds particularly when gauging the thickness of an epithelial band which

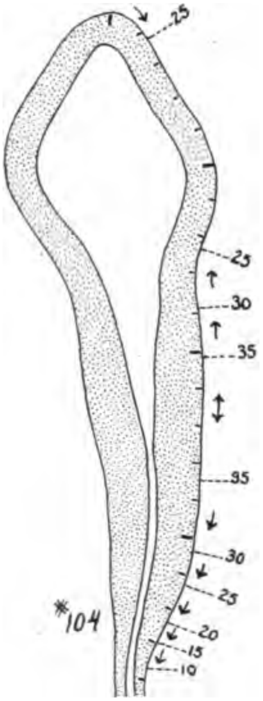

Fig. 2 Drawing of a transverse section of the archenteron of a Torpedo embryo 4.0 mm. long (H.E.C. 765) showing the method of measuring sections for reconstruction by Weber's method. The short lines extending into the section mark the centimeter segments used in eliminating lateral curvature from the section. The longer lines extending out to the right from the section are the gauge lines. The figures at the end of the gauge lines indicate in micra the thickness of the epithelium at the points touched by them.

is rapidly changing in caliber, or when gauging a portion of a band which forms a sharp curve. Failure to observe this precaution causes noticeable error both in the gauge readings and in the position of the gauge points on the drawing. There will often be encountered considerable portions of the wall, the thickness of which corresponds exactly to the gauge unit or a multiple of it. My rule, made arbitrarily, has been to mark as the gauge point the first (i.e., most dorsal) level at which such a zone is encountered.

A reconstruction outline is now made on section-lined paper in the usual manner, except that only the dorsal margin of the gut is mapped out. Using this margin line as a base, the transverse section lines are divided into centimeter segments corresponding to those made on the margins of the drawings of the sections. In practice it is convenient to mark the point separating each block of 5 cm. segments in a different color to aid in plotting. The ventral margin of the gut and the gauge points, which have been determined on the cross section drawings, are now plotted on the transverse lines of the reconstruction in the usual manner, except that instead of measuring the distance of each point from the dorsal margin of the section and transferring this measurement to the corresponding section line as is customary, one measures the distance of the point from the nearest centimeter mark in each case. The ventral outline of the reconstruction and the gauge lines indicating the thickness of the epithelium are established by connecting all the points of the same order as is done in an ordinary graphic reconstruction. The reconstruction will now have the form seen in figure 3. There the base and transverse reconstruction lines are lightly drawn and the centimeter segment points are indicated as small dots on the latter. The outline of the reconstruction is indicated in heavy line. The ventral margin posterior to the anterior intestinal portal has been cut away arbitrarily in a straight line. The gauge lines indicating the thickness of the epithelial wall of the organ are represented in heavy broken line. The vertical figures placed at the termination of the gauge lines indicate their values in micra.

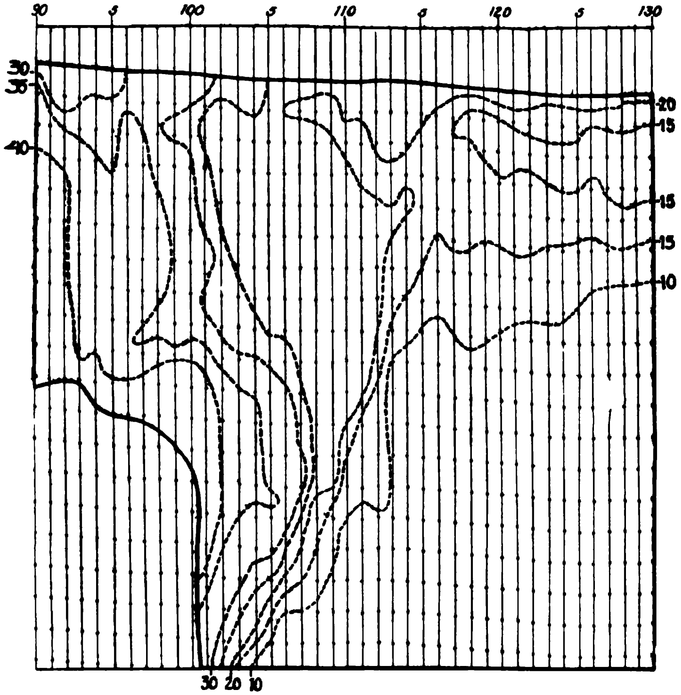

Fig. 3 Reconstruction plat of a Weber's reconstruction (lateral view) of the portion of the archenteron bounded by the lines *A* and *B* in figure 1. The light vertical lines represent the transverse sections. The outline of the reconstruction is represented in heavy solid lines. The gauge lines bounding areas of epithelium of different thicknesses are represented in heavy broken lines. The dots on the transverse lines represent the boundaries of the centimeter segments described in the text and shown in figure 2. The vertical figures at the margins of the reconstruction give the value of the gauge lines in micra. The figure is reduced to one-half the size of the original reconstruction, which was made at a magnification of 350.

There remains but to color in the areas which are marked off by the gauge lines. Weber did this with water-color and secured excellent results, as his figures show, but a much simpler and quite as satisfactory a method is to use papers of different shades of gray for the different areas. Such papers should be as near 'pure' mixtures of black and white as it is possible to secure. The only kind which I have found satisfactory is the 'Herring' series which has been prepared for color work in psychological laboratories. The shades numbered 3, 4, 7, 10, 16, 20, 30, 42 and 46 make a satisfactory series of marked but fairly equal gradations.

To build up the final colored reconstruction gray papers are selected, equal in number to the areas mapped off on the reconstruction by the gauge lines. The entire outline of the reconstruction is now traced upon the lightest colored paper. This area is cut out and pasted firmly upon a piece of compo or plaster board. Upon the paper of the next darker shade there is traced an outline similar to the preceding except that the space representing the thinnest area is cut away. This second sheet is pasted upon the first one so that the similar angles and sides correspond. In this way the area of thinnest epithelium is represented by the lighter colored paper and all thicker areas by the darker one. This process is continued, all thinner areas being cut away from each new outline until the darkest. and final shade of paper will have the shape and will represent the area of the thickest epithelium only. This method of building up the papers of different colors in strata will be found much easier than to cut out each separately and then attempt to fit them together in a mosaic. Figure 4 is a half-tone made directly from such a colored reconstruction and based upon the plotting illustrated in figure 3.

The example just described is of a lateral view reconstruction. Reconstructions may be made by Weber's method to show dorsal or ventral views of epithelial structures as well. For this purpose the transverse sections of the structure are drawn and measured in the same manner as that described. A reconstruction plat is then laid out as follows. A vertical line is drawn in the middle of the sheet to represent the dorsal median line of the structure and transverse section lines are drawn on either side at the proper distance apart and at right angles to the median vertical one. The centimeter points, which on lateral view reconstructions must be measured off on each transverse section line, can be located on the plat in this case by simply ruling vertical lines parallel to and at centimeter intervals from the median one. The gauge points and lines marking the boundaries

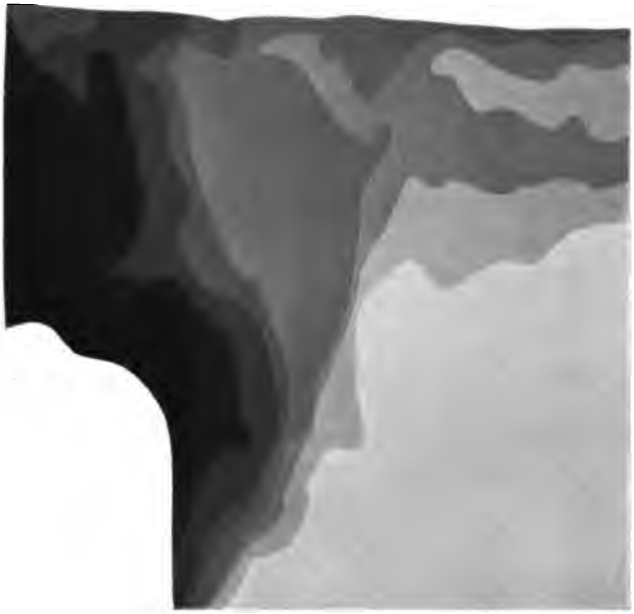

Fig. 4 A finished Weber's reconstruction (lateral view) made from the plat represented in figure 3 and including that part of the archenteron lying between the lines *A* and *B* in figure 1. The thicknesses of the epithelium in several parts of the reconstruction are represented by the various shades of gray. Starting with the lightest, these several shades represent the following epithelial thicknesses; (1) below 10μ; (2) 10 to 15μ; (3) 15 to 20μ; (4) 20 to 25μ; (5) 25 to 30μ; (6) 30 to 35μ; (7) 35 to 40μ; (8) above 40μ. This figure is reduced to three-fifths the size of the original reconstruction.

between areas of different epithelial thickness are mapped out as in the lateral-view reconstruction. Should the reconstruction be of a tubular structure, the ventral median line must be determined in each cross section drawing. In reconstructing, the tube is then represented as split along its ventral median line and flattened out laterally; i.e., the lateral margins of the reconstruction represent in fact the ventral median line of the original tube. Figure 5 is an example of such a reconstruction plat made from the same object employed for the lateral view reconstruction already described. The method of representation and lettering are the same as used for figure 3.

Several of the possible uses and advantages of this method of reconstruction have been mentioned. It remains to speak of a few disadvantages and sources of error. In the first place the outlines of the reconstructions, aside from the one used as a base line, are not strictly such as would be secured by the ordinary graphic or plastic methods. In eliminating the lateral curvatures from the transverse sections, the dimensions of the figure are increased dorso-ventrally or laterally, as the case may be, without a similar increase antero-posteriorly. Weber made no attempt to eliminate the curvature seen in cross sections, regarding it in fact as of some value in indicating contour. I have already pointed out the disadvantage in reconstructing without making this correction, which I think should be done even at the expense of some accuracy of outline, which can easily be determined by the other reconstruction methods.

While the error introduced by this method is small when dealing with structures having surfaces approximating those of cones or cylinders, it is considerable when applied to spherical surfaces. Spherical surfaces do not admit of being spread out into planes as do those of cones and cylinders. Cartographers, who have much this same problem in representing large areas of the globe, have developed a number of methods of projection to meet, in part, this difficulty. These are, however, too complex for our work and are all based upon representations of the perfect sphere. For the purpose at hand surfaces which approximate spherical ones can best be treated by first compensating for the vertical curvature by the method of segmenting the outline of the section already described; and, second, by allowing for longitudinal or horizontal curvature by increasing the distance separating the cross section lines on the reconstruction plat. The latter can be done by determining the actual length of the outline of the structure to be reconstructed and dividing this distance by the number of sections which the structure contains. Multiplying the figure thus secured by the magnification at which the reconstruction outlines are drawn gives one the distance which should separate the section lines on the reconstruction plat. This practice differs from that of ordinary reconstruction in

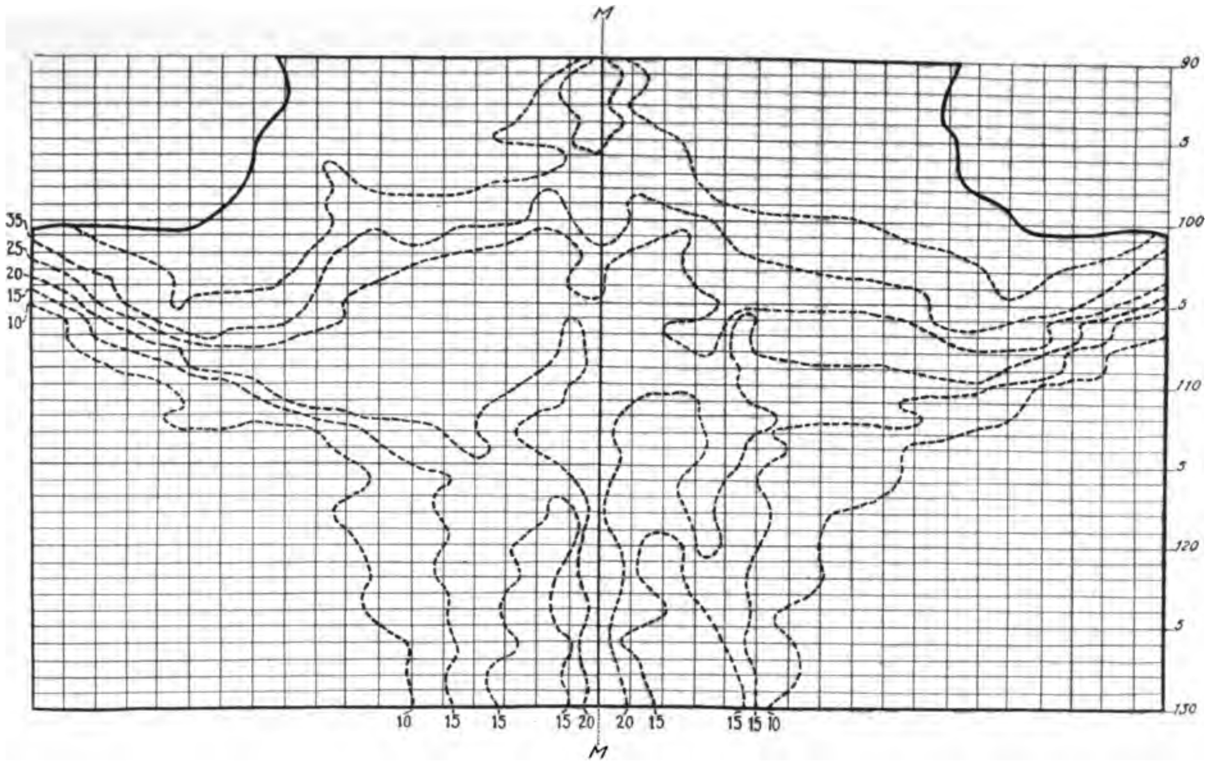

Fig. 5 Reconstruction plat of a dorsal view reconstruction of the same object as shown in figures 1, 3 and 4. The line *M-M* represents the dorsal median line of the archenteron. The lighter lines parallel to it are the boundaries of the centimeter spaces. Scheme of drawing and lettering corresponds to that of figure 3. The scale of magnification is the same as for figure 3.

that the longitudinal axis of the structure is used in the customary method instead of the actual length of the outline as in this case. Reconstructions made with these corrections will show approximately the area and shape of any given outline upon a curved surface, although neither will be strictly accurate. As a rule such correction will be unnceessary unless one is dealing with surfaces which curve very abruptly. Measurements of the thickness of epithelial plates which curve longitudinally or horizontally will always be a little exaggerated because such plates are cut somewhat obliquely by transverse sections. There seems to be no practical way of eliminating this error.

Finally, the reconstruction will represent the variations in thickness of the epithelium as occurring in definite steps and not as gradual transitions as in nature. Most of this artificial distinction can be eliminated by using the smallest unit of measurement practicable and thus increasing the number of shades present in the reconstruction while decreasing the degree of their difference. Great reduction of figures in their reproduction also aids in securing the effect of gradual transition.

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THE HISTOGENESIS OF THE SELACHIAN LIVER

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FORTY-FIVE FIGURES (SEVEN PLATES)

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INTRODUCTION

The structure of the adult selachian liver is far removed from that type which is generally considered characteristic of the organ in vertebrates. This variance from the common vertebrate type is seen in the great accumulation of fat in the hepatic cells, in the comparatively slight development of the bile duct system and in the absence of lobulation of the kind generally found in higher vertebrates.[1] However, these pecularities which distinguish the selachian liver are not manifested until a comparatively late stage in the development of the organ. In earlier stages characters common to the liver in all vertebrates, but which are often masked or modified in higher forms, are shown with unusual clearness. It is chiefly with these more fundamental characters such as the formation and anastomoses of the hepatic cylinders, the differentiation of the minor bile ducts and the relation of the parenchymatous and vascular structures in the liver, that this paper has to do. The specific characters of the selachian liver, which have been mentioned, have been considered only incidentally.

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[1] The histology of the adult elasmobranch liver was first briefly described by Leydig ('51) from observations on Chimaera. He published a more complete account dealing with several forms of selachians in 1853. Later descriptions of the general histology of the adult liver have been given by Shore and Jones ('89), Pilliet ('90), and Holm ('97). Deflandre ('05) has investigated the fat content of the hepatic cells, and Monti ('98) has studied the bile capillaries by the Golgi method.

The main material employed in this study consisted of embryos of Squalus acanthias, but specimens of Raia batis, Torpedo ocellata, Mustelus canis, Mustelus laevis and Squatina angelus have been used for supplemental and comparative work. For a large part of the Acanthias material, and for the specimens of Mustelus laevis, I am particularly indebted to the late Dr. Charles S. Minot, who not only permitted the removal of numerous series from the Harvard Embryological Collection, but also provided special material for use in this work.

DEVELOPMENT OF THE HEPATIC CYLINDERS

1. *Literature*

Our conception of the glandular structure of the vertebrate liver rests upon a large number of observations made mainly in the first half of the last century and culminating in the work of Eberth ('66) and Herring ('72). Since that time it has been recognized that the liver is a compound gland with a more or less regularly branching system of ducts and with a terminal network of anastomosing end pieces, and our knowledge of the details of this network has been greatly extended by the Golgi method in the hands of Retzius ('92), Hendrickson ('96) and others.

It is generally stated that the anastomotic type of liver is a modification of the compound branching gland type. Of this the best proof is the phylogenetic one, for in the lower cyclostomes, the liver is indeed a true compound branching gland, as has been shown by the work of Retzius ('92), Holm ('97), Cole ('13) and others. In Petromyzon, however, anastomoses occur occasionally in the embryo (Brachet '97) and are very numerous in the adult (Renaut '97). On the side of embryology little evidence of this modification has been offered, although the statement that the liver arises in many vertebrate embryos as a branching gland, and that it takes on its adult reticular structure through the anastomoses of its end pieces, is common enough in texts. But an examination of the literature shows no more complete account of this early period of the histogenesis of the liver than that given by Remak ('55) excepting Hilton's work ('03) on the pig which has hardly received the attention that it deserves. The study of the actual course of the anastomosis of the end pieces has also been neglected, and the only clear statement to be found regarding this process is not in connection with the liver at all but in Laguesse's ('96) description of the histogenesis of the pancreas where he found numerous anastomoses at different stages.

The literature of the histogenesis of the liver in selachians is scanty and has been to a large extent reviewed in the comprehensive works of Oppel ('00) and Fiessinger ('11).

Balfour's ('76) account was the first to appear and as it has formed the basis of all later descriptions, it is given here in full:

> By stage K the hepatic diverticula have begun to bud out a number of small hollow knobs. These rapidly increase in length and number and form the so-called hepatic cylinders. They anastomose and unite together so that by stage L there is constructed a regular network. As the cylinders increase in length their lumen becomes very small but appears never to vanish.

Hammar ('93) in the course of a study devoted particularly to the development of the larger hepatic structures illustrates the development of the hepatic cylinders in two models of the liver of Torpedo embryos corresponding in development to Balfour's stages L and M. He makes no comment upon them aside from stating that the trabeculae increase in number and decrease in caliber in the period intervening between the two stages.

Brachet ('95) studying Torpedo embryos determined with accuracy the area of the hepatic diverticulum which gives rise to the hepatic tubules, and confirmed Balfour's description of the structure of the hepatic cylinders in later stages.

Holm ('97) figured and described very briefly sections of the liver of two Scyllium embryos of advanced stages.

Most of our later information concerning the embryonic hepatic parenchyma comes from the studies of Braus ('96) on embryos of Acanthias, Spinax and Scyllium. Confirming Balfour's observations in regard to early stages he noted a complete and regular anastomosis of the tubules in Acanthias embryos of 38 mm. Here the tubules were of even size and consisted in cross section of seven cells surrounding a lumen of variable size but distinctly larger than that seen in the adult. The hepatic cells of this stage were free from fat. In older embryos of Spinax and Acanthias the cells were fat laden. Braus saw no side branches nor blind endings of any hepatic capillaries.

Choronshitzky ('00), studying Torpedo, found several secondary hollow outgrowths from the hepatic pouch in his "Stadium II," which corresponds approximately to Balfour's stage K, and to the Normal-plate Nos. 22–24. In "Stadium III" which is represented by considerably older embryos, the liver pouches give rise to a number of small hollow buds, the cavities of which communicate with that of the pouch. "Die Leber macht im allgemeinen den Eindruck einer verzweigten Drüse." By "Stadium IV" the hollow buds have been transformed into much branched hepatic trabeculae which contain no traces of lumina. Choronshitzky's opinion of the mechanical influence of the blood vessels on the formation of the hepatic tubules will be discussed in a later part of this paper.

Minot ('00) in discussing the development of sinusoids, mentions the presence of the first short hepatic cylinders in an Acanthias embryo of 11.5 mm. and speaks of their anastomosis and growth in older stages. He noted the interesting retardment of development which is to be seen in later stages in the caudal tip of the liver as compared with the cardiac end.

Debeyre ('09) made use of observations upon the development of the hepatic cylinders in Acanthias to lay the ghost of the theory of the mesodermal origin of the hepatic parenchyma, which had been again raised a short time before by Géraudel ('07). He gives no complete or detailed history of the cylinders but notes, with illustrations, their general appearance in embryos 16, 22, and 30 mm. in length, respectively. In the latter he recognized the beginnning of a period of pronounced increase in the diameter of the cylinders. Debeyre noted the presence of numerous granules in the apices of the hepatic cells and bases upon this the interesting suggestion that the liver may serve as an organ of internal secretion during a part of embryonic life.

2. Early development of the hepatic tubules

In this account the structures which have been variously termed hepatic cords, trabeculae, cylinders and tubules will be spoken of as tubules as long as they remain as portions of simple or branching unanastomosed glands. The term hepatic cylinders will be employed for the same structures after the process of anastomosis has taken place.

The exact time when the anlagen of the hepatic tubules first make their appearance is somewhat variable. In general they are first to be seen in embryos from 7.5 to 9 mm. in length, being somewhat younger than Balfour's stage K[2] and corresponding to numbers 22 and 23 of the Normal plate series. Such embryos have from fifty to sixty-five segments and four or five pairs of gill pouches of which the anterior three or four may open externally. The spiral valve is in the process of formation, making at this time one or two complete turns of the intestine and the vitelline duct is reduced to a short wide canal. The form of liver anlage at this stage is represented somewhat diagrammatically in figure 1. The organ consists of a ventral median pouch from the foregut just anterior to the vitelline duct. The anterior part of this pouch is full and rounded and may be termed the pars hepatica mediana. From the median pouch spring two large lateral pouches which form together the pars hepatica lateralis. In stages just preceding the appearance of the tubule anlagen the lateral pouches are smooth and globose and project outward almost at right angles to the median hepatic pouch. At the time when the tubules are formed, however, the lateral pouches are flattened transversely and have entered upon a pronounced dorsal growth. Connected with the liver pouch above and in front and with the anterior wall of the yolk stalk behind is a small thick walled sac, the anlage of the gall bladder. The hepatic tubules take origin entirely from the pars hepatica mediana and the pars hepatica lateralis. These areas are indicated in stipple in figure 1.

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[2] In correlating embryos with stages of Balfour's series only the general development of the embryo has been considered and not the state of development of the organ under discussion.

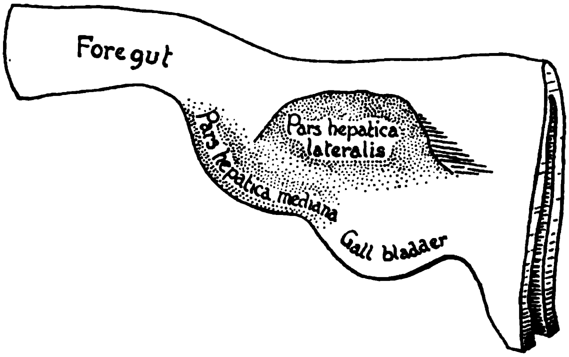

Fig. 1 Lateral view of a semi-diagrammatic reconstruction of the foregut and liver of an Acanthias embryo 9 mm. long. The areas represented in stipple give rise to hepatic tubules.

The tubule anlagen arise in two forms: as slight longitudinal ridges upon the lower part of the outer surface of the lateral pouches and as very small irregularities of the dorsal margins of the same structures. When first observed the longitudinal ridges are two to four in number on either side. They extend almost the entire length of the lateral pouches and are distinctly separated by shallow lateral furrows. Sometimes these ridges may be subdivided longitudinally at their ends. Figure 17 is a view from the left side and below of the liver of an embryo 7.5

mm. in length (S.C. 14).[3] This specimen bears two ridges on the left hepatic pouch, but the right pouch is entirely smooth. The irregularities of the margin of the hepatic pouches, which also form tubules, are at first so ill defined as to be scarcely noticeable unless reconstructed. Then it is seen that the formerly straight dorsal margin has a wavy contour.

The tubule ridges rapidly increase both in size and number. New ridges appear ventral and mesial to the earlier ones and extend to the base of the lateral pouches and upon the ventral surface of the pars hepatica medialis of the median liver pouch. No new ridges appear dorsal to the first ones and I think that no new ridges arise between older ones. By the time the embryo reaches a length of 10 to 12 mm. each lateral pouch wall and lateral half of the pars hepatica medialis bears seven to ten tubule ridges. This is the total number formed on either side, and when tubules thereafter arise from the pouch directly, they do so as individual tubules and not in the form of tubule ridges. Figure 18 is a view from the left side and below of a reconstruction of the liver of an Acanthias embryo 10 mm. in length (S.C. 20) showing the later form of the tubule ridges and the beginning of the differentiation of tubules from them.

Before all the tubule ridges are formed the ones which first develop are broken up by transverse or oblique furrows into rather irregular rows of low mound like elevations. These elevations are the anlagen of the individual hepatic tubules and may be seen in figure 18 referred to above. They are semicircular or nearly so in cross section and nearly twice as long as broad, their greater length being always directed antero-posteriorly. Almost immediately the tubules begin an active outward growth and each is differentiated into a distal extremity which often is large and pouch like, and a more slender proximal stalk which is connected with the hepatic pouch and which is circular in cross section. The further growth of the tubules takes place by the formation of tubules of the second order from the distal expansions of the primary ones. The secondary tubules are short conical projections rarely over one and one-half times as long as their greatest diameter. They arise from the sides of the distal expansion and almost always extend out at right angles to the axis of the primary tubule. Those primary tubules which arise from the sides of the hepatic pouches and are packed in among their fellows commonly assume a T-shaped form with the limbs of the T directed antero-posteriorly. The primary tubules which arise from the margins of the hepatic pouches become somewhat larger than those formed from the tubule ridges and give rise to from three to seven secondary tubules from their expanded distal chambers. These secondary branches may again subdivide into branches of the third order and upon these in turn there may occasionally be found nipple-like projections which represent the fourth order of tubules. In the large majority of cases, however, anastomosis takes place before tubules of the fourth or even the third order are formed. Figures 19 and 20 are of wax reconstructions of tubules from the lateral hepatic pouch of an embryo 13.3 mm. in length (S. C. No. 18). In figure 19 one sees the beginning of the outpouching of the distal expansion into secondary tubules. These structures are well marked projections in the older tubule shown in figure 20. Figures 22 and 23 are two views of a hepatic tubule from the dorsal margin of the left hepatic pouch of an embryo 15 mm. long (H.E.C. 227). The latter figure shows tubules of the fourth order. Figure 21 is a wax reconstruction of two young primary tubules from the ventral surface of the pars hepatica medialis of the same 15 mm. embryo. One of these shows the beginning of tubules of the second order.

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[3] In designating embryos the following abbreviations will be employed: H.E.C., Harvard Embryological Collection; K.U.E.C., Embryological Collection, Department of Zoology, University of Kansas; S.C., author's collection.

When the tubule ridges first appear on the hepatic pouches they are due to the increased thickness of the epithelium in these places and not to an evagination of the pouch wall. In cross sections of the ridges (see fig. 28, a cross section of the lateral wall of the left hepatic pouch of an embryo of 8 mm. K.U.E.C. 542) it is noticeable that the nuclei which in other parts of the wall are arranged in two interlocking rows tend to be reduced to a single row which lies near the external surface of the epithelium. There seems to be but a single layer of very high columnar cells at this point but the cell boundaries are not very clear. There is always a broad clear zone of cytoplasm towards the lumen of the pouch opposite the tubule ridge. Mitotic figures do not occur in the ridges but are frequent in the zones of epithelium between them. Following the formation of the ridge there appears a very shallow trench on the internal surface of the epithelium. The lumina of the individual tubules appear as the tubules themselves are differentiated through the breaking up of the ridges into rounded anlagen. One then finds in each tubule anlage a narrow slit-like cleft passing between the cell walls at right angles to the pouch cavity. This is shown in figure 29. Thus at first the tubule cells do not lie at right angles to the tubule lumen but nearly parallel to it. Later the cells assume an oblique position (see again fig. 29) and finally come to lie in the typical radiating position in relation to the lumen (fig. 30). As the tubules develop the cells become shorter and their nuclei change from elongately ovoid to nearly spherical bodies.

3. *The anastomosis of the hepatic tubules*

The liver is transformed from a gland of the branching to one of the reticular type by the anastomosis of its end pieces. The process begins in Acanthias in embryos from 12 to 15 mm. in length. Such embryos have from 70 to 85 pairs of somites and correspond roughly with Balfour's stages L and K and with Nos. 25–26 of the Normal plate series. The tubules arising from the dorsal portions of the lateral liver pouches precede in their differentiation those of other regions and it is generally among these dorsally placed tubules that anastomoses are first found. Later the tubules of the lower parts of the lateral pouches and finally those of the pars hepatica medialis enter upon the process. Variants from this general plan of procedure are not uncommon. Figure 2, of a sagittal section of the left hepatic pouch of an embryo 14 mm. long (S.C. 30) shows how general anastomoses are when once they begin in a given region.

No particular degree of differentiation seems necessary before a tubule takes part in an anastomosis, and when this process begins in any region both branched and simple tubules fuse indifferently. The commonest form of anastomosis is that established by the end to end fusion of tubules, but tubules may also join end to side or side to side. The last type is much commoner in later stages when more opportunities are offered for this form of contact. Anastomoses also seem to take place with equal frequency between tubules formerly quite separate or between minor branches of the same tubule when it is possible for such branches to come in contact.

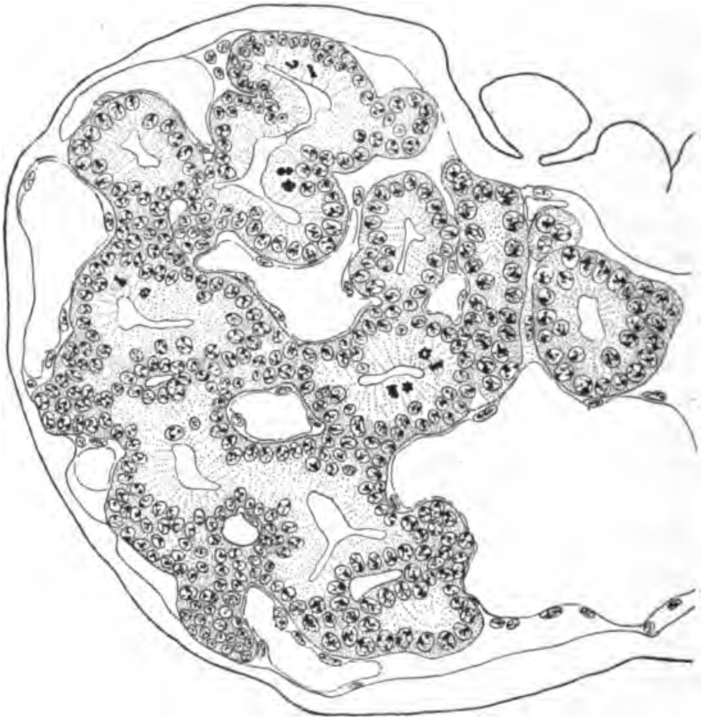

Fig. 2 Sagittal section of the anastomosing tubules of the left hepatic pouch of an Acanthias embryo 14 mm. long (S.C. 30.) × 100.

Figure 24 is of a wax reconstruction of three anastomosing tubules from the left hepatic pouch of an embryo 15 mm. in length (H.E.C. 227). The pouch wall from which the tubules spring is cut away squarely around the base of each tubule. Tubules *A* and *B* are completely anastomosed end to end but there is still a distinct constriction at the plane where they have joined and the lumen within is considerably narrower than in the bodies of the tubules. Although not clearly shown in the figure both *A* and *B* are T-shaped and in each case the other tip of the tubule ends freely without anastomosis. Tubule *C* is a compound one and the subdivision which passes over to join with *B* is a branch of the third order. There is as yet no actual anastomosis between *C* and *B*, but the epithelial walls of the two are in direct contact.

The lack of any account of the histology of anastomosis seems to warrant a rather full description of the process here. The process may be rather arbitrarily divided into three steps or stages. In the first step the tubules come in actual contact; in the second there occurs the fusion of their walls and a rearrangement of the cells forming them, and in the third there is an establishment of a connecting channel between the two original lumina.

These stages of anastomosis can be observed in the liver of Acanthias embryos of any length from 13 to 45 mm. They are more easily followed, however, in young specimens. The figures which are used here to illustrate the process have been taken from embryos under 20 mm. in length. In each case the sections have been followed through and the tubules involved have been reconstructed to make sure that the picture presented was not due to an oblique plane of section or a misinterpretation of a segment of a single complicated tubule.

Early stages of contact of the anastomosing tubules are illustrated by figure 30 which is a transverse section of the left hepatic pouch of an embryo 13.3 mm. long (S.C. 18). Numerous tubules extend outward from the lateral wall of the hepatic pouch. Of these the larger number are still in the form of simple tubules which are expanded distally but a few have entered upon anastomosis. The blood vessels pass among the tubules covering them in part with a thin film of endothelium which is interrupted at some places by the attachment of strands of mesenchyma and at others by the apposition of the distal surfaces of tubules with the insheathing layer of splanchnic mesothelium. Whether this endothelial covering forms an absolutely continuous partition without fenestra between the blood and the tubule epithelium cannot be definitely determined without injected specimens, but there seem to be places where the separation is not complete. Anastomosis is inaugurated by the contact of the involved tubules. At first a few mesenchymal or endothelial cells may separate the tubules but these are apparently pressed to either side so that the entodermal cells soon lie in actual contact. Often at first only four or five tubule cells actually meet, but shortly there is formed an area of contact which generally is not quite so large as the caliber of either tubule involved. Sometimes the cells of one tubule indent the wall of the consort but this is not the common rule. There are no basement membranes about the hepatic tubules, but the basal margins of the cells seem a little thickened so that for a time after contact the line between the cells of the two anastomosing tubules is still distinct. With the disappearance of this line the tubules may be considered as fairly fused.

The connection between the two fused tubules is often drawn out a little forming a short stalk or bridge between them. This will be termed here the connecting stalk. An indentation upon the external surface of this stalk indicates the line of fusion of the tubules. The connecting stalk, when present, is generally a little less in diameter than are either of the tubules and the boundaries of the cells forming it are not clear. One can follow the cells, however, by the position of their nuclei. Although at first sight the nuclei appear scattered without order in the stalk or at the point of anastomosis, a little study enables one to determine with some accuracy which nuclei are contributed by each tubule. The two rows of nuclei approach one another and may interlock but their radial arrangement in regard to the lumina of the two original tubules is not lost at first. They then pass to one side or the other of the connecting stalk, leaving a clear cytoplasmic core in which cell boundaries are faintly distinguishable. In so doing the axes of the nuclei rotate through an angle of 90° so that instead of being parallel to the long axis of the connecting stalk as at first, they are now at right angles to it. Such rotation can be determined, of course, only in young tubules where the nuclei are oval and not circular in section. Figure 26, drawn from an embryo 19 mm. in length (S.C. 3), shows an early step in these changes. Figure 3 A is a graphic reconstruction of the tubules shown in cross section in figure 26. In this section the rows of nuclei belonging to the two tubules involved are distinguishable although the process of migration of the nuclei to the sides of the connecting piece is clearly under way. In figure 36 of a later stage from an embryo 14 mm. in length (S.C. 30), all the nuclei with the exception of one have passed to the sides of the connecting stalk.

In following the course of the nuclei in tubule anastomosis one is but tracing the movements of the cells in which they are contained, for it is hardly to be considered that the nuclei shift their axes within the cells, and moreover the few faint cell boundaries which may be made out show the same changes in position as do the nuclei. The hepatic cells of the connecting stalk have shifted through an arc of about 90° and when a lumen is established through the center of the connecting stalk it is bounded at least in greater part by the same cell surfaces which were presented to the lumina in the original tubules. In other words, while the tubule cells involved in anastomosis have shifted in position, their surfaces and their axes will bear the same relation to the new lumen which they did to the former one. Their long axes will be at right angles to the lumen while the inner and outer surfaces of the cell remain constant in both the original position in the simple tubule and the later position in the anastomotic segment. The polarity of the cell, in the sense of the term as used by Rabl ('88, '90), is not disturbed by anastomosis.

The lumen of the anastomosis is formed by clefts which extend out from the lumina of the formerly simple tubules. These clefts are at first small and irregular. They pass between the rather irregular borders of the radially arranged cells of the connecting stalk or plate, and, meeting, become confluent. It is not until some time after these clefts have joined that the lumen of the anastomosis acquires its full size and regularity. A late stage in the establishment of this connecting channel is shown in figure 27 from an embryo 19 mm. long (S.C. 3). Here the clefts are still separated by a single cell. Figure 3 B is a graphic reconstruction of the tubules involved in this anastomosis.

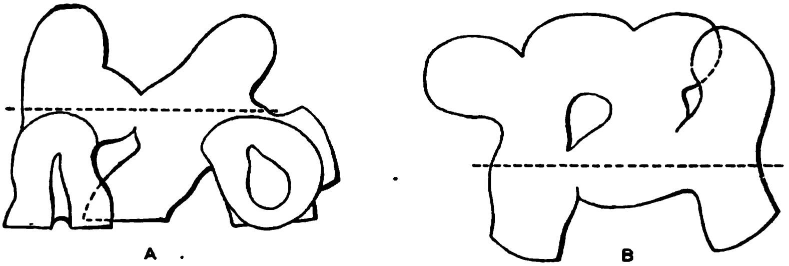

Fig. 3 Graphic reconstructions of anastomosing hepatic tubules in Acanthias. Detailed drawings of the sections indicated with dotted lines will be found in plate 2. *A*, tubules from an embryo 19 mm. long (S.C. 3) (see fig. 26). *B*, tubules from an embryo 19 mm. long (S.C. 2) (see fig. 27). × 100.

4. Later history of the hepatic cylinders

a. Growth of the hepatic network. Immediately after anastomosis has occurred the liver increases in size very rapidly. At first this growth is due almost entirely to the increase in number of the hepatic cylinders, but later the greater portion is brought about by the tremendous enlargement of the hepatic sinusoids. After anastomosis new cylinders are added to the existing network in three ways: by the formation of tubules from the remains of the hepatic pouches, by the formation of blind sprouts or buds from the sides of cylinders forming the network and by the production of new cylinders at the periphery of the network from the cylinders located there. These methods of addition to the hepatic network cease in the order given and the proportional amount contributed to the network by the several methods is in inverse order to that in which they are listed above.

The direct production of new hepatic cylinders from the hepatic pouches contributes but little to the bulk of the hepatic network and continues for only a short period after anastomoses are formed. As described in section 2, there are numerous tubules which arise at the bases of or between tubules which have previously sprung from the hepatic pouches. These younger tubules are at first small nipple shaped elevations arising singly and not from ridges. So long as the earlier tubules remain simple the later tubules follow them closely in their development, but when anastomoses become common among the older tubules and the sinusoidal circulation is well established, the younger tubules anastomose almost immediately after their formation either with one another or with cylinders of the already established network. In later stages hepatic tubules may sometimes arise as loops, the ends of which are attached to the wall of the hepatic pouch. By the time the embryo reaches a length of approximately 20 mm. (Normal plate Nos. 27–28, Balfour's stage N) the hepatic pouches are transformed into veritable hepatic ducts and thereafter no new hepatic tubules arise from them.

Hepatic cylinders, as has been seen, give rise to secondary buds while still in the form of single tubules, and this budding process continues long after anastomosis. The process can be most clearly demonstrated by the means of thick sections of which figure 41 from an embryo 24 mm. in length is an example.

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[4] The method of preparation of the thick sections used in this study was as follows: The embryo was infiltrated with celloidin and cemented to a piece of infiltrated spleen or liver which in turn was fastened to a fiber block. The specimen was then cleared by Gilson's method, which makes the block almost transparent. The block was then placed in the microtome clamp and a strong beam of light from a condenser directed upon the object. A Greenough binocular microscope equipped with low power lenses was set up over the object. With this arrangement it was possible to follow in detail the process of section cutting. Sections were then carefully cut away until the exact region desired was reached. By focusing with the binocular it was possible to determine the approximate thickness of the section needed to just include the desired structures, and this section was then removed with a single cut. Sections made in this way are often superior to reconstructions for the study of the form of very small structures. They are best stained in a very dilute carmin solution and cleared for a long period in cedar oil, after which they can be observed with the binocular microscope, or better with the aid of an Abby binocular eyepiece.

In the area of approximately 1 mm. represented here there are five blind buds projecting from the cylinders into the sinusoids. This method of addition to the network continues for a considerable period. I have found no traces of new buds in the body of the liver after the great increase in size of the cylinders when the embryo reaches a length of about 40 mm. However, in the portions of the liver which are the last to be formed, i.e., the dorsal margins and the posterior tips of the lateral lobes, this method of cylinder formation continues until the embryo reaches a length of 50 to 60 mm.

While in the earlier stages of the development of the hepatic network the increase comes perhaps equally from peripheral and interstitial growth, in later stages the latter method is by far the more important. The hepatic network terminates peripherally in a large number of blind knobs which by their growth and division give rise to a large amount of hepatic tissue. The cells of these terminal knobs remain in a comparatively undiffentiated condition while those of the more central part of the network are undergoing rapid changes in structure. Figure 43 shows a small portion of the tip of the lateral lobe of an embryo 20 mm. long. The mesothelium covering the liver has been removed. Here the terminal knobs are seen projecting from the general network and are often attached to the mesothelial sheath by strands of mesenchyma. This specimen was prepared by cutting a thick celloidin section of the region desired by the method already described. The celloidin was then dissolved away and the mesothelial covering stripped from the fragment with fine forceps.

The peripheral addition to the hepatic network takes place at first over the entire surface of the liver. But like the interstitial method of addition it is later limited to the tips of the lateral lobes and to the dorsal margins of both the body and lateral lobes of the liver which at a comparatively late stage grow rapidly upward between the stomach and the lateral body walls. In embryos 60 mm. in length these areas are much restricted and they cannot be seen in an embryo 80 mm. long.

In the rapidly growing parts of the liver the cylinders often terminate peripherally in expanded end-bulbs or vesicles which contain a lumen many times the diameter of that of the typical cylinder. Such vesicles are found in embryos from 25 to 40 mm. in length but not in older specimens. Their walls consist of a low columnar or cuboidal epithelium. Figure 4 is a cross section of one of these structures from the lateral lobe of an embryo 36.6 mm. long (S.C. 10). The significance of these vesicles is unknown to me.

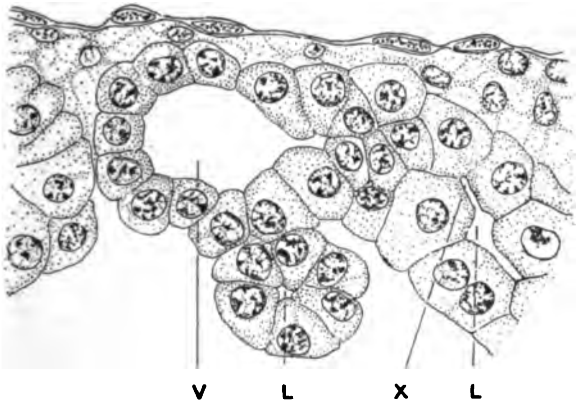


Fig. 4 Terminal vesicle of a hepatic cylinder from the lateral lobe of an Acanthias embryo 36.6 mm. long (S.C. 10). × 400. *L*, lumen of hepatic cylinder; *V*, terminal vesicle; *X*, side branch from central lumen of cylinder.

b. Changes in the size and structure of the hepatic cylinders. When the hepatic tubules enter upon anastomoses they are irregular in form and of variable caliber. The lumina of the tubules are large and irregular and are generally surrounded in cross section by 14 or 15 cells, if one may judge from the number of nuclei present, for cell boundaries are often indistinct at this time.

Table 1 shows some of the changes which take place in the course of the later development of the cylinders. The measurements and cell counts given in this table are in each case averages determined from twenty fair cross sections of cylinders of the posterior part of the median lobe of the liver. The cross sections of cylinders were taken at random from this region, except that those lying in the rapidly growing peripheral zone were avoided in each case.

In the first specimen of the series the number of cells bounding the lumen in cross section averages 12.5. The diameter of the tubules has become more uniform and averages 58 micra. Examples of such tubules are illustrated in figure 31. At about this time the liver begins to increase greatly in size. This growth is due in part to the actual increase in number of hepatic cells, as is indicated by the presence of numerous mitotic figures in the hepatic cylinders, but a much greater part of the increment is due to the establishment of the huge hepatic sinusoids. With this increase in size of the sinusoids the tubules are distinctly reduced in size, their average diameter dropping from 58 micra in an embryo 15 mm. in length to 37.6 micra in one 25 mm. and 35.6, in one 28 mm. in length. This reduction in diameter may be due somewhat to the decrease in the size of the lumen, but is caused mainly by the actual decrease in the number of cells surrounding the lumen at any one plane. The process is a continuous one after anastomosis is established, but is more rapid

TABLE 1

Measurements of the hepatic cylinders in Acanthias embryos

LENGTH OF EMBRYO IN MM.	AVERAGE NUMBER OF CELLS IN CROSS SECTION OF TUBULE	AVERAGE DIAMETER OF TUBULES IN MICRA
15.0	12.5	58.0
20.6	10.0	50.0
25.0	8.0	37.6
28.0	7.0	35.6
33.0	6.6	37.0
37.0	6.0	38.5
47.0	5.2	40.0
50.0	4.6	46.0
60.0	5.3	39.0
80.0	5.0	43.0
95.0	3.7	54.0

at first, dropping from an average of 12.5 in an embryo of 15 mm. to 7 in one of 28 mm. I think that this rapid and early reduction is due to the stretching of the tubules caused by the dilatation of the sinusoids among them. The tubules are attached to each other and to the mesothelial wall of the liver by strands of mesenchyma which may aid in the process by pulling upon the cylinders as the mesothelial wall is rapidly stretched in all directions. These mesenchymal strands can be seen in figure 43 of the liver of an embryo 20 mm. long.

After the embryo reaches a length of from 28 to 30 mm. the hepatic cylinders again begin to increase in size. They rise rapidly in diameter from an average of 35.6 micra in an embryo 28 mm. long to 46 micra in an embryo of 50 mm. and 54 micra in an embryo of 95 mm. This growth is still noticeable in an embryo of 220 mm. in which, however, the cells were too collapsed to admit of accurate measurement. While the diameter of the tubules increases, the size of the lumen and the number of cells surrounding it at any given plane as steadily decreases. Thus the average number of cells seen in cross section of a cylinder at 25 mm. is 8, at 50 mm. 4.6, and at 95 mm. 3.7. This indicates that the increase in size of the cylinder is due to the growth of the individual cells forming it and not to their multiplication, as is the early increase in size found in embryos from 13 to 16 mm. long.

Almost all this growth is due to the deposition of fat in the hepatic cells, the nuclei of which remain stationary or actually decrease in size. While the cells begin to increase in size even when the cylinders are becoming reduced in caliber, due to factors already mentioned, this growth is not sufficient to make up for the reduction until the embryo reaches a length of more than 30 mm.

The thick sections illustrated in figures 40, 41 and 42 show graphically some of the changes just described. Figure 40 is a section of a liver in which the process of anastomosis is well under way. It shows the large size and irregular caliber of both the cylinders and their lumina. Figure 41 is from a specimen in which the hepatic sinusoids have nearly reached their highest development and in which the cylinders are reduced to slender tubes. Figure 42 is from a somewhat older specimen in which the cylinders have again begun to increase in diameter. The changes in the number of cells surrounding the lumen of the cylinder in cross section are shown in the four figures forming plate 3.

My remarks upon the changes in the finer structure of the hepatic cells can be regarded as little more than notes made in the course of the general study of the growth of the cylinders.

During the development of the hepatic tubules and cylinders, the nuclei of the cells forming them are modified in shape, size and structure. At the time when the tubule ridges first appear upon the hepatic pouches the hepatic nuclei are elongately oval in outline, their longer axes averaging from 14 to 15 micra and their lesser axes from one-third to one-half of this length (fig. 28).[6] With the definite outpouching of the hepatic tubules the nuclei become broader and shorter, but their volume remains practically unchanged (figs. 29, 30). In an embryo 10 mm. in length (S.C. 20) from which tubules have been described and figured in the preceding part of this paper, the hepatic nuclei average 8 micra in diameter and 11 micra in length. Anastomosis has apparently no effect upon either the size or structure of the nuclei. In embryos from 10 to 20 mm. long one can follow the change in shape of the majority of the nuclei from broadly oval to spherical bodies. In a 20.6 mm. embryo (H.E.C. 1494, fig. 32) the great majority of the nuclei are spherical and have a diameter of 10 micra. Thereafter they gradually decrease in size even as the cells grow in size through an increase in fat content. In an embryo 47.3 mm. long (S.C. 11) the average diameter of the nuclei is 7.5 micra and in one of 95 mm. (H.E.C. 1882) it is little if any less. As the fat accumulates in the liver cells the nuclei may again change in shape, being in many cases pressed against the margin of the cell and assuming an oval or crescentic outline suggesting the form of the nuclei found in true fat cells. The nuclei which remain spherical have an average diameter of 7 micra or a little less (in embryos 200–240 mm. long).

[6] All measurements given here were made from paraffin sections.

In early stages the nucleus is always in the basal portion of the cell. After anastomosis they are found more centrally located. In later stages they are found scattered, sometimes in the center of the cell surrounded with a film of protoplasm from which threads extend to the cell periphery; often close to the lumen wall of the cell and sometimes near some other point in its periphery.

Before the tubule anlagen appear the hepatic nuclei contain one, two or three large masses of chromatin which surround nucleoli and are generally applied closely to the nucleus wall. These chromatin masses are round or oval in shape and smooth in outline except for one or two small chromatin threads which may extend outward from each mass. The remaining space of the nucleus is filled with a clear nuclear sap through which run a few delicate and faintly staining fibrils. Such nuclei are characteristic of the young Acanthias embryo being found also in the cells of the mesenchyma, mesothelium, walls of the medullary canal and in the mesonephric tubules and duct. Those of the mesenchyma have been fully described by McGill ('10) in a study of the development of the striated muscle of the oesophagus in the dogfish. The figures in Neal's ('14) recent work illustrate the similar structure of the nuclei in the nervous system. Figure 28 shows the structure of a number of these nuclei stained with iron-hematoxylin. As the hepatic cells are differentiated the chromatin masses above mentioned become more irregular and there are given off from them a number of chromatin strands which eventually form a coarse network. The chromatin masses are reduced in size and may become detached from the nuclear wall. They also may be somewhat broken up and present a granular appearance. On the other hand, the chromatin threads which have originated from them may fuse forming secondary and generally smaller karyosomes which do not surround nucleoli. These changes are shown in figures 31 to 34, 38 and 39. The adult hepatic nucleus is rich in chromatin which is arranged in a coarse network containing several karyosomes some of which are probably the remains of the original ones and some of which are the result of secondary aggregation of chromatin granules from the chromatin threads.

Most of these karyosomes are applied to the nuclear wall. The hepatic cell retains the typical embryonic nuclear structure much longer than do the cells of the mesenchyma, mesothelium, nervous system or urogenital system. This typical embryonic structure is lost first in the cells forming the gall bladder and major hepatic ducts, next in the minor hepatic ducts which are formed from cylinders already well started upon a development towards typical hepatic parenchyma, and finally from the hepatic cells proper.

As has been remarked, fat droplets as indicated by vacuoles in the protoplasm of the hepatic cells appear, when the embryos obtain a length of about 25 mm. The use of special reagents would no doubt demonstrate the presence of fat prior to this stage. The droplets are found at first at the base of the cell, but later, in embryos 65 to 95 mm. long, droplets are found scattered through the entire cell body reducing the protoplasm to the network which has been described for the adult hepatic cell of selachians by Shore and Jones ('89) and Pilliet ('90).

In Acanthias embryos about 30 mm. long the gall bladder begins to press against the hepatic tissue which lies on either side and above it. This process is probably brought about by the great growth of the internal yolk-sac which lies below the gall bladder. This pressure of the gall bladder causes some degeneration of the hepatic tissue immediately surrounding it. Toldt and Zuckerkandl ('78) have described a similar process in the human embryo.

5. *Development of the hepatic tubules in other forms of selachians*

The development of the hepatic tubules in Torpedo, Raia and Mustelus differs somewhat from that of Acanthias. In these forms the lateral hepatic pouches do not reach the great development found in Acanthias and tubules are formed from these structures at a comparatively earlier stage. The omphalomesenteric veins are somewhat larger than in Acanthias and at the time when the individual hepatic tubules develop veinous channels are found both medial and lateral to the hepatic pouches.

The tubules which are formed from the dorsal parts of the pouches and of the pars hepatica medialis arise singly or in small clusters as do those in the same situation in Acanthias, but in the forms under discussion the dorsal or marginal tubules form a much greater part of the whole number produced than is the case in Acanthias. In Torpedo and in Mustelus the tubules from the ventral portion of the lateral pouches arise as in Acanthias from tubule ridges. These ridges are but slightly elevated and the corresponding grooves on the internal surfaces of the pouches are wide and shallow. The individual tubules which form from these ridges do not remain as mound-like structures arranged in rows but grow out almost at once and like the dorsal tubules appear as slender tubes extending outward between the veinous sprouts. In most cases the hepatic tubules which are first formed come in contact with the splanchnic mesothelium covering the liver.

The omphalo-mesenteric veins in these forms increase in size very rapidly and before tubule formation has progressed very far the anterior, ventral and lateral surfaces of the liver pouch are practically surrounded by a venous lake. All tubules which arise after this period project into this sinus and in their growth they push its endothelial wall before them. This process is illustrated in figure 44, which is of a thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The number of tubules arising directly from the pouch walls is small in these forms as compared with Acanthias for the surface area of the pouches is much reduced from the first. The large majority of the later tubules is formed by the branching of the earlier ones.

In Acanthias the hepatic tubules in the earlier period of their development are short, broad and pouch-like and the branches which arise from them are nipple-like projections. In the other forms studied both the primary and secondary tubules are slender elongated tubes which are early united in a complex branching and anastomosing network, the meshes of which are separated by sinusoids of large size. Figure 44 shows an anastomosis of a young hepatic tubule in Mustelus, and figure 45 of a similar section of an embryo about 4 mm. longer of the same species shows the complete establishment of the hepatic network.

While the early tubules of Raia, Mustelus and Torpedo are smaller in cross-section than are those of Acanthias, the lumina of these tubules are considerably larger. The cells lining the lumina are cubical or low columnar in outline as compared with the high columnar type found in Acanthias. The nuclear structure is quite similar to that of the hepatic cells in Acanthias, the chromatin covered nucleoli described for that form being particularly prominent in Torpedo and Mustelus.

The later history of the cylinders is quite similar to that of Acanthias. The cylinders rapidly increase in diameter and the contained lumina become smaller. The increase in diameter of the cylinders is due, as in Acanthias, to the growth of the individual cells, and the number of cells about the lumen in any given section steadily decreases. The nuclear changes are similar to those found in Acanthias. In such specimens of Mustelus and Squatina as I have examined the fat contained in hepatic cells remains in discrete droplets instead of forming one large mass as is generally the case in Acanthias. The same is true to some extent of Torpedo (fig. 35).

Pilliet ('90) has described areas of comparatively undifferentiated cells in the adult selachian liver. These cells form the portions of the cylinders which lie about the hepatic-portal veins. They are of comparatively small size and contain centrally placed nuclei which stain deeply with alum-carmin. The fat content of the cells is less than of those cells located elsewhere, and particularly of those lying in the neighborhood of the hepatic veins. Pilliet regards these smaller cells as reserves or nests of young cells which, from their position near the nourishing vessels, contribute to the growth of the organ. Apparently he did not find them in all the specimens which he examined.

I have seen no evidence of a retardment of the differentiation of the cells near the hepatic-portal veins in Acanthias embryos, and if such occurs it must be at a late period in the development of the organ. There are numerous small cells in the immediate neighborhood of the hepatic-portal vessels, but they all form portions of the terminal bile ducts, the development of which will be described in a later part of this paper. Such embryos of Torpedo as I have examined agree with Acanthias in lack of any definite nests or reserves of undifferentiated cells about the hepatic-portal veins. In well advanced Mustelus embryos, however, the hepatic cells which surround the larger branches of the hepatic-portal veins do remain somewhat smaller than those of the remainder of the liver and are not so completely charged with fat.

In summary it may be said that the chief differences between the two types of selachian liver, that represented by Acanthias and that by the several other forms mentioned, lies almost entirely in the earlier stages. These differences seem to be dependent on the difference in the size and arrangement of the omphalomesenteric veins at the time of the formation of the hepatic tubules.

DEVELOPMENT OF THE MINOR RAMI OF THE HEPATIC DUCTS

The formation of the minor rami of the hepatic ducts is closely associated with the history of the hepatic cylinders. In a preceding publication (Scammon '13) it was stated that all of the major and some of the minor rami of the hepatic ducts arise from the constricted bases of certain fairly definitely placed clusters of hepatic tubules. In following the history of these tubule clusters it was found that they become separated from the hepatic pouch from which they arise by a broad and rather indefinite peduncle which is at first hardly more than an extension of the pouch wall. Later this peduncle becomes constricted and elongated, forming a small branch from the pouch which by this time is transformed into a segment of the hepatic duct. This development begins before tubule anastomosis gets fairly under way and continues at the time when anastomoses are taking place. No new major rami arise as outpouchings after anastomosis is established and aside from the actual lengthening of already established rami, which is not great, all further growth of these structures takes place by the transformation of pre-existent hepatic tubules into ducts.

This illustrates well the two methods of hepatic duct formation. The first and more primitive type is that suggested by Minot ('93, p. 763) in which the duct is the result of a direct outpouching of the wall of the hepatic diverticulum. In the second and specialized type the duct is produced by the transformation of portions of the network of hepatic cylinders. Selachians clearly stand near the bottom of the scale in this phase of development. Here the ductus choledochus, the hepatic ducts and their major rami arise as outpouchings and only the minor rami and the most distal portions of the major ones are of trabecular origin. In ganoids, amphibians, reptiles and birds the ductus choledochus and the proximal part, at least, of the hepatic ducts are formed by outpouching and the remainder of the duct system from trabeculae. In the mammals apparently the ductus choledochus alone is the result of an outpouching, but further investigation may change this conception.

The differentiation of hepatic cylinders into bile ducts is very closely associated with their relations to the blood vessels. Bile ducts are only formed from cylinders which are in contact with the main trunks of the hepatic-portal veins or their larger and more definite branches. Still more striking is the fact that the side of the cylinder towards the vessels precedes in a very marked degree the differentiation of the opposite side and in fact in the smallest ducts the cells of the opposite side of the cylinder may never be transformed into duct epithelium at all but complete the ordinary development of true hepatic cells. Such a terminal duct from the liver of an embryo 95 mm. in length is shown in figure 37. On the other hand, of the many cylinders which closely surround the vascular trunk only a very small percentage is transformed into ducts. The development of the minor ducts is extremely small in proportion to the amount of hepatic parenchyma, smaller, I think, than in any other group of vertebrates. There is absolutely no indication of any system of intercalated ducts.

In the differentiation of a hepatic cylinder into a bile duct the former first approaches more nearly a perfect circle in cross section and the lumen distinctly enlarges. The cells on the vascular side of the lumen are reduced in actual height but become more columnar in form because of the still greater reduction of the size of their bases. At the same time the nuclei which in hepatic cylinders are round or broadly oval in section and lie near the center of the cells become elongately oval in outline and tend to retreat to the bases of the cells. The cells are so diminished in size that the nuclei which increase little, if any, in bulk almost fill them. The nuclei lose their typical structure of a clear karyoplasm containing one or two large chromatin masses from which radiate chromatin threads and present instead a reticular chromatin network made up of evenly distributed granules of about the same size. The protoplasm becomes homogeneous and colors darkly with plasma stains. An example of such a developing duct at an early stage is shown in figure 39. Approximately one-third of the duct which abuts upon a blood vessel shows considerable progress in differentiation, while the cells of the segment opposite it are true hepatic cells. Between the two are zones of transitional cells. To the side of this duct is a smaller one in still an earlier stage of differentiation. The outline sketch shown in figure 5 illustrates the changes in shape of the hepatic cells at the time of duct formation.

The cells of the large hepatic ducts which are formed from the hepatic pouches and their evaginations show much the same steps in cytomorphosis as do those just described. The nuclei of the larger ducts, however, are oval at the start and so undergo no changes in form, but the change in chromatin arrangement is the same as in the minor ducts. In the gall bladder the same changes also take place but at a late stage (60–80 mm.) the nuclei again become circular in cross section and come to occupy the centers of the cells which are much elongated.

It is sometimes stated that the bile duct epithelium is formed of cells of a more primitive type than those of the cylinders or trabeculae.. In the forms under discussion, however, the bile duct cells have departed farther from the embryonic type than the parencyhmal cells, if we may judge by their nuclear structure.

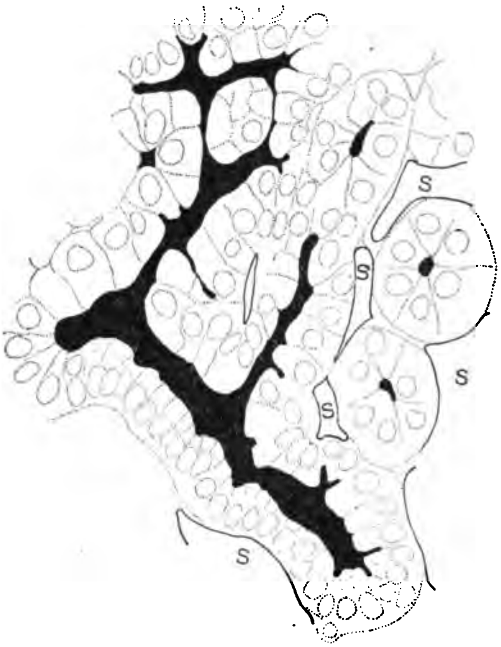


Fig. 5 Minor hepatic duct with connecting hepatic cylinders from the median lobe of the liver of an Acanthias embryo 37 mm. long (H.E.C. 353). × 400. Lumina of hepatic duct and cylinders solid black; hepatic cells in stippled outline; sinusoids, *S*, in unbroken outline.

DEVELOPMENT OF THE HEPATIC MESENCHYMA

It is well known that the mesenchymal tissue of the selachian liver is extremely scanty in the embryo although fairly abundant around the blood vessels in the adult. No particular study has been made of its origin, but Minot ('01) in his study of sinusoids, and more recently Debeyre ('09) in following the development of the hepatic cylinders, have noted the occasional delamination of mesenchymal cells from the mesothelium covering the liver and the presence of such elements along the walls of the hepatic sinuses. Debeyre also figures a cross section of a small portion of the periphery of the liver of an Acanthias embryo 30 mm. long, which shows small spurs of mesenchymal tissue extending inward from the mesothelium between the hepatic cylinders. The origin of the hepatic mesenchyma can be followed with little difficulty in Acanthias. Here it appears that this material is probably derived completely from the mesothelium but at two distinct periods and from two different regions, and that in both cases this proliferation is associated with distinct irregularities of splanchnic mesothelium.

The first mesenchymal proliferation appears at a little later time than the formation of the stroma of that portion of the gut which lies posterior to the liver. In Acanthias embryos 5 mm. in length the liver pouch is but slightly differentiated and consists of two shallow diverticula which lie in the lateral walls of the archenteron and are fused anteriorly to form the median hepatic pouch. The archenteron of this region is clothed on either side by a layer of splanchnic mesoderm which is continuous above with the radix mesenterica and below with the splanchnic layer of the blastoderm. At the radix this layer is much thickened but it is reduced to a moderately thin layer over the sides of the archenteron. The irregular endothelial walls of the omphalomesenteric veins intervene between the ventral part of the archenteron and the mesothelium, but no mesenchyma is present. Irregular processes from the mesothelium, however, do extend inward and in places it appears as though cells were about to be delaminated. A short time later the hepatic pouch increases much in size and extends anteriorly far in front of the anterior intestinal portal. With this change the ventral parts of the investing layers of splanchnic mesothelium are brought in contact and eventually fuse, thus forming for some time a ventral mesentery. In connection with this process there appear two distinct sets of mesothelial irregularities. These consist of the mesothelial villi on the right side and of numerous irregular folds on the left.

The mesothelial villi found in connection with the covering of the selachian liver were first described by Choronshitzky ('00), although they were observed in other forms long before that time. Hochstetter ('00) has given their later history in Acanthias in connection with his study of the formation of the septum transversum. As Hochstetter has stated, these structures are found only on the right side. They appear in embryos of 45 to 30 segments as a thickened plate of the mesothelium overlying the right omphalo-mesenteric vein (fig. 6 A). This plate is soon thrown into a series of pouch like irregularities, and the spaces thus formed are filled with a delicate network of protoplasmic processes from the mesothelial cells. The cores of the villi are thus at no time really empty, and are soon occupied by delaminated mesenchymal cells. From the bases of these villi mesenchymal cells are proliferated apparently both from the walls and the mesenchymal core, and soon come to form a small mass on the right side lying just below the omphalo-mesenteric vein. On the opposite side the irregularities are not villi but longitudinal folds. Unlike villi they do not arise from a thickened plate, the mesothelium at this point remaining as thin as elsewhere at the time of formation. Afterward the cells become more columnar and a mass of mesenchyma, larger but similar in other respects to that of the opposite side, is proliferated and underlies the left omphalo-mesenteric vein. Figure 7 shows the position and extent of these two mesenchymal proliferations in an embryo 7.5 mm. long (H.E.C. 1496). Soon after this stage the ventral mesentery breaks down and the ventral surface of the liver is free throughout its extent. With this process there is a delamination of mesenchymal cells along the median ventral line which unites the lateral ones already described, and there is thus formed a general ventral bed of mesenchyma which, as growth proceeds, forms a coating about the gall bladder and constitutes a large loose-meshed mass which extends forward from the gall bladder to the anterior mesothelial wall of the liver.

This constitutes the first and ventral contribution of mesenchyma to the liver from its mesothelial envelope. In its entire extent it produced the mesenchyma which surrounds the gall bladder, and later the vessels adjacent to it, the covering of the cystic duct, and to an undeterminable degree the sparse mesenchymal tissue of the lower part of the hepatic parenchyma. The irregularities of the mesothelium on the right side do not continue for any great period. By the time the embryo reaches

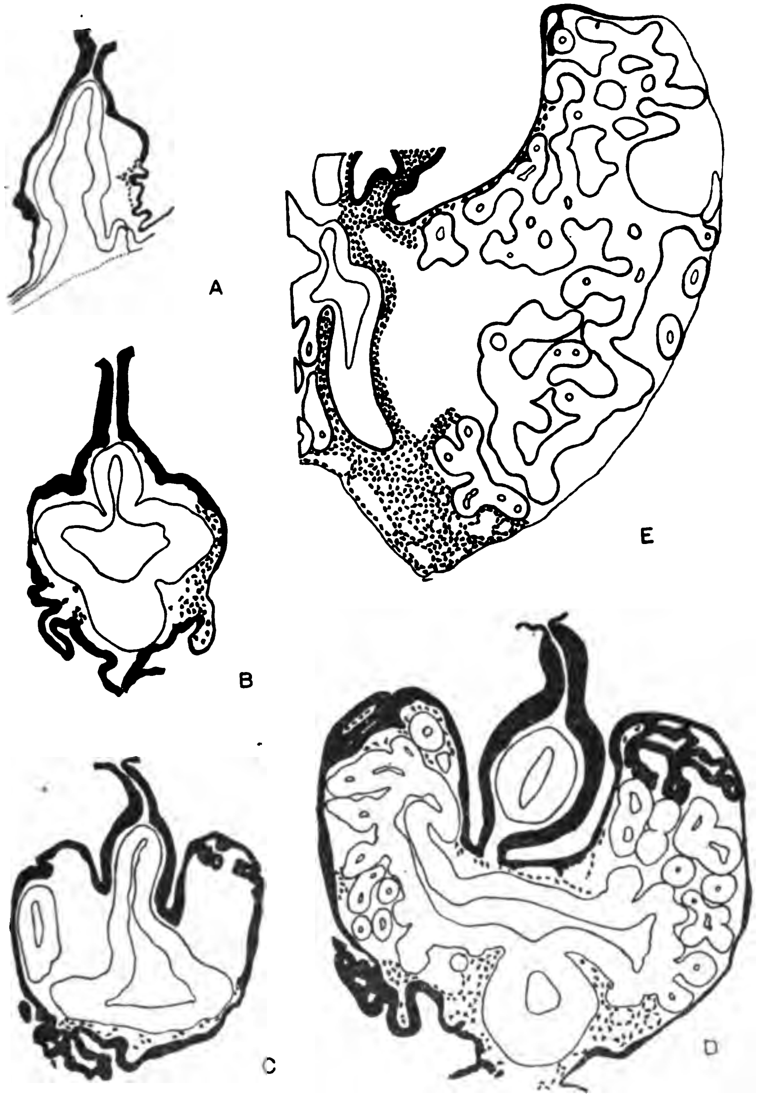


Fig. 6 A series of transverse sections of liver anlagen of Acanthias embryos of different ages. The mesothelium is represented in solid black, the mesenchyma in coarse stipple and the entodermal structures in outline; the blood-vessels are omitted. *A*, embryo 6.4 mm. long (S.C. 19); *B*, embryo 9 mm. long (H.E.C. 1495); *C*, embryo 10 mm. long (C.S. 20); *D*, embryo 15 mm. long (H.E.C. 1494); *E*, embryo 20.6 mm. long (H.E.C. 1494); all × 50.

a length of 15 mm. the mesothelium is smooth and much reduced in thickness. When the embryo reaches a length of 18 mm. the villi are reduced to small conical projections and are absent in embryos of 20 mm. Thereafter the mesothelium covering the ventral part of the liver on both sides becomes steadily reduced in thickness, and in embryos of 25 mm. and longer it is an exceedingly thin layer of squamous epithelium.

The second and dorsal mesenchymal proliferation begins at a later stage than does the ventral one. In an embryo 6.4 mm. in length (S.C. 19) (fig. 6 A) the dorsal portion of the mesothelium is seen to be slightly thinner than that of the ventral zone. As the lateral pouches of the liver are pushed upward, the omphalomesenteric veins come to lie mainly dorsal to these structures and the mesothelium over them is gradually thickened. This thickening is most noticeable at the lateral margins of the dorsal surface (fig 6 B). Soon after, in embryos of 10 to 12 mm., the mesothelium of this region becomes distinctly thickened in places and is invaginated forming tubules the walls of which are continuous with the splanchnic mesothelium and the lumina with the coelomic cavity (fig. 6 C and fig. 7). Often these tubules first appear as long trenches on the coelomic surface of the mesothelium. The sides of these trenches coalesce, thus roofing over the depression and forming tubules which open into the coelom at both ends. Other tubules grow in and end blindly. Examples of these early tubules are shown in figure 8. Although fairly regular at the start, these structures soon become irregular in caliber and form, and very often anastomose. Their lumina may be occluded in places thus forming mesothelial cysts which are generally connected by solid stalks with the covering mesothelium. At the time of their highest development the tubules form an anastomotic network which fills the dorsal fifth of the anterior part of the liver and they are then easily mistaken at first sight for true hepatic tubules (fig. 6 D). More careful study shows a number of differences between the two structures. Aside from the nuclear differences to be mentioned later, the mesothelial tubules are more irregular than the hepatic cylinders, their walls are thinner both actually and relatively as compared to their lumina, the cells are more columnar and there is no definite external cuticula as there seems to be in the hepatic cells at this time. The cytoplasm of the cells is also more granular. The mesothelial tubules may extend downward and come in contact with the hepatic cylinders, but generally they are replaced in this region by cords of mesenchymal cells. Figure 9 is a cross-section of a portion of the liver of an Acanthias embryo 15 mm. in length (H.E.C. 227) showing these tubules (*Mes.t.*) in the highest state of development.

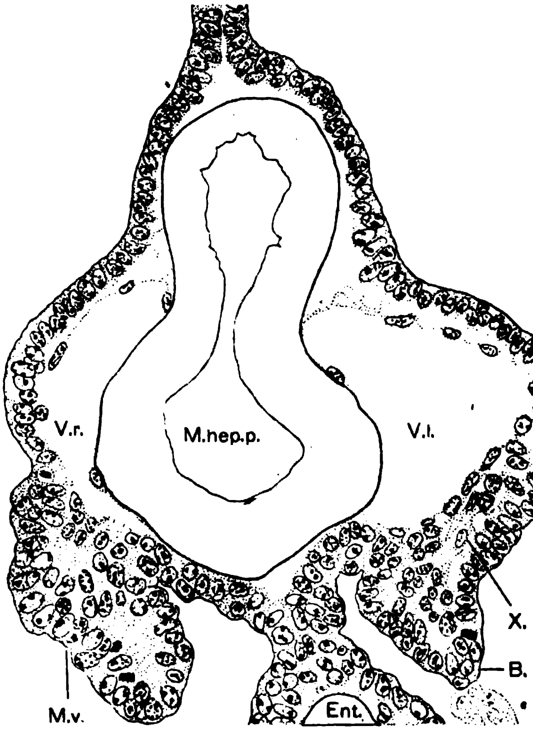
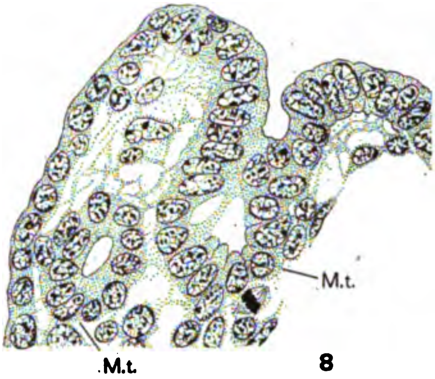


Fig. 7 Transverse section through the anterior part of the anlage of the liver of an Acanthias embryo 7.5 mm. long (H.E.C. 1496). × 400. *B*, tangential section of the mesothelial wall surrounding radicle of the left omphalo-mesenteric vein; *M.hep.p.*, median hepatic pouch; *M.v.*, mesothelial villus; *V.l.*, left omphalo mesenteric vein; *V.r.*, right omphalo-mesenteric vein; *X*, proliferated mesenchyma.



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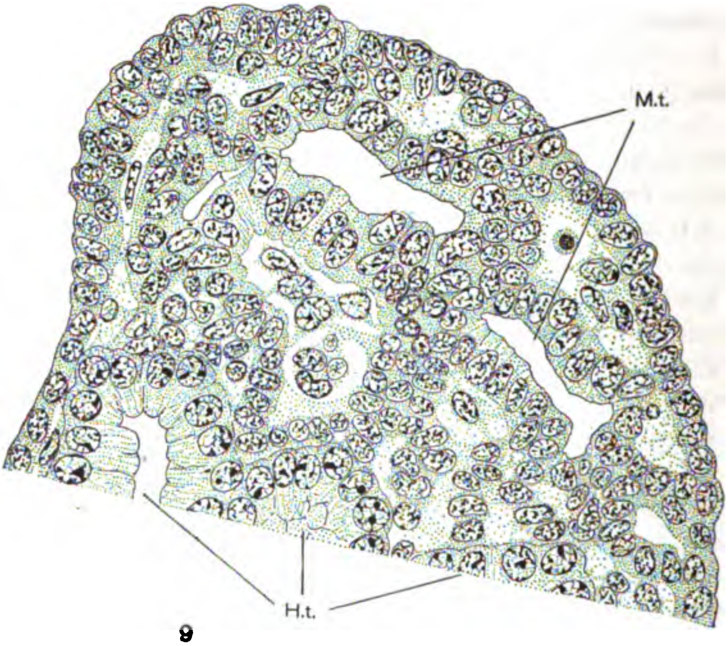

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Fig. 8 Transverse section of the mesothelial covering of the dorso-lateral margin of the liver of an Acanthias embryo 13.3 mm. long (S.C. 18). × 400. *M.t.*, mesothelial tubules.

Fig. 9 Transverse section of the dorsal portion of the liver of an Acanthias embryo 15 mm. long (H.E.C. 227). × 400. *H.t.*, hepatic tubules; *M.t.*, mesothelial tubules.

The mesothelial tubules do not remain long as such. The lumina are soon obliterated and the solid cords thus formed break up into mesenchymal strands. This process begins at the inner end of the cords and can be seen in figure 9. By the time the embryo reaches a length of 23 to 25 mm. the cords are entirely replaced by such mesenchymal strands which run in among the tubules which have now invaded this region (fig. 38). At 28 to 30 mm. even these strands have entirely disappeared and mesenchymal cells can be found only occasionally. The formation of mesenchyma by tubular ingrowths of mesothelium does not preclude the occasional delamination of single cells from the splanchnic mesoderm direct, but this delamination contributes but little to the total of the hepatic mesenchyma.

The nuclei of the splanchnic mesothelium in embryos 5 or 6 mm. in length, before mesenchymal delamination begins, are broadly oval in outline. They have the typical structure which is found in the nuclei of most of the tissues at this time. Each nucleus contains two or three large masses staining black with Heidenhain's hematoxylin. Each of these masses consists of a nucleolus surrounded by a thick layer of chromatin. The remainder of the nucleus is filled with a clear karyoplasm with a few faint strands of chromatin. These nuclei have been well described by McGill ('10) who studied the mesenchyma of the foregut region in the dogfish. As cells are delaminated from the mesothelium and as the mesothelial tubules are broken up into a mesenchymal syncytium, most of the nucleoli disappear and the chromatin is gradually scattered in a finely divided network. Similar changes take place in the nuclei of mesothelial cells but at a later period. A comparison of figures 7 and 38 will show the two extremes of this change. Mitotic figures are numerous in the mesothelium but scarce in the mesenchyma derived from it.

The relation of the mesothelial tubules to the sinusoids is of interest in the light of Bremer's ('14) recent work on the earliest blood vessels in man. Bremer found mesothelial cords from the extra-embryonic coelome connected with the angiocysts and solid cords of the vascular net in the body stalk, and he suggests that the elements of the vascular net arise from these ingrowths.

I have not found that the cavities in the mesothelial tubules or cysts of the selachian liver connect with the blood spaces. As the walls of these structures break up into mesenchymal strands it is impossible with ordinary section and staining methods to distinguish between them and the endothelium surrounding the adjoining sinusoids. This is particularly true if one tries to follow the mesenchymal strands which penetrate between the hepatic cylinders in later stages such as the one shown in figure 38. The mesenchymal and endothelial cells form free anastomoses. McGill ('10) apparently found the same condition in a much younger embryo, judging from figure 9 of her paper with which the section shown in my figure 7 is in agreement.

The reduction in the thickness of the mesothelial covering of the liver and the disappearance of the mesothelial villi is no doubt due in part to the great growth of the contents of the former, particularly of the hepatic cylinders and sinusoids. This increase takes place rather suddenly in embryos from 18 to 20 mm. in length and the reduction in the thickness of the mesothelium is to some extent coincident with it. It is noticeable, however, that in many cases when a hepatic cylinder comes in contact with the covering mesothelium the latter layer is distinctly reduced in thickness, its constituent cells change from a high columnar to a cuboidal form and the nuclei from oval to nearly spherical bodies. This change is probably not due to pressure for in places where two tubules come in contact with the mesothelium and are separated by a distance hardly equal to their own diameter the mesothelium becomes thin at the points of contact and remains thickened over the small intervening portion. Were the effect of tubule contact only a matter of pressure, one might expect some flattening of this intervening place as well. Also the mesothelium is not pushed outward at these points of contact but remains on a level with the surrounding surface.

One is tempted to suggest the homology of this dorsal mesenchyma-producing mass with the 'Vorleber' of Kölliker and His. But this similarity is only a superficial one as the mass just described does not appear until after the liver is well developed, while in birds and mammals the 'Vorleber' is a precursor of that organ. Also the selachian mesenchymal mass lies in the dorsal and posterior part of the liver along its free upper surface, while the 'Vorleber' lies anteriorly and is broadly connected with the dorsal wall of the coelom. Ziegler ('88) showed long ago that the mesenchyma of the intestine in selachians arose from two longitudinal zones of proliferation from the splanchnic mesoderm. One of these zones lies above the archenteron and the other ventral to it. The method of development of the hepatic mesenchyma indicates that these dorsal and ventral zones of proliferation extend forward into the liver region as well and remain distinct for a considerable period even after the liver has separated from the gut above it. A comparison of a semi-diagrammatic figure of a cross-section through the hepatic region with Ziegler's diagram of a similar section through the middle of a trunk segment shows the essential agreement in the origin of the splanchnic mesenchyma in the two regions (fig. 10).

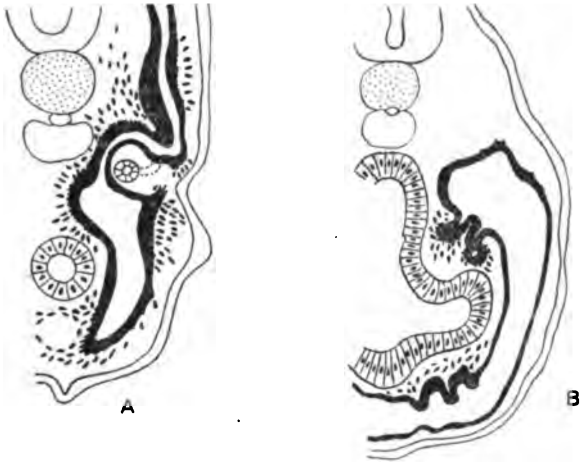

Fig. 10 A comparison of Ziegler's figure: *A*, of the mesenchymal tissue in the trunk region of a selachian embryo with a semi-diagrammatic figure; *B*, showing the origin of the mesenchyma in the hepatic region of an Acanthias embryo.

HISTORY OF THE HEPATIC SINUSOIDS

The sinusoids of the selachian liver are most striking objects and at one time form decidedly more than half of the entire bulk of the organ. Minot ('00) has given the only description of them which is at all complete as a part of his exposition of the nature of the sinusoidal circulation. Brief notes are also to be found in the papers of Holm ('97) and Debeyre ('09). The general development of the veins of the liver in these fishes has been studied by Rabl ('92), Hoffmann ('93) and Hochstetter ('93). The early development of the omphalo-mesenteric veins has been observed in detail by Mayer ('87) and by Ruckert ('88). Therefore I shall give only the briefest outline of the general history of the veins, and will begin with the conditions found in Acanthias embryos from 8 to 10 mm. in length. In such embryos there are paired omphalo-mesenteric veins extending forward to the sinus venosus on either side of the gut. The left vein is somewhat larger than the right. At first the right and left trunks are quite separate but soon they form two anastomoses, one posterior to the pancreas and the other, a little later, just posterior to the liver below the foregut. From the latter anastomosis the veins pass forward on either side of the foregut and medial to the lateral hepatic pouches until they reach the anterior end of the liver where they join and form the sinus venosus. It is important to note that in Acanthias in early stages only the medial surfaces of the lateral pouches are in contact with vascular channels. There are no vascular channels between the lateral surfaces of the hepatic pouches and the mesothelium covering them.

Figure 11 A shows a reconstruction of a somewhat later stage in which important changes have taken place. Now only the left omphalo-mesenteric vein passes through the groove between foregut and lateral hepatic pouch to join the sinus venosus. The right vessel ends blindly anteriorly and is connected with the left by the large anastomosis which lies behind the liver. In the meantime either a single vessel or several vascular sprigs have grown back on either side from the sinus venosus and passing

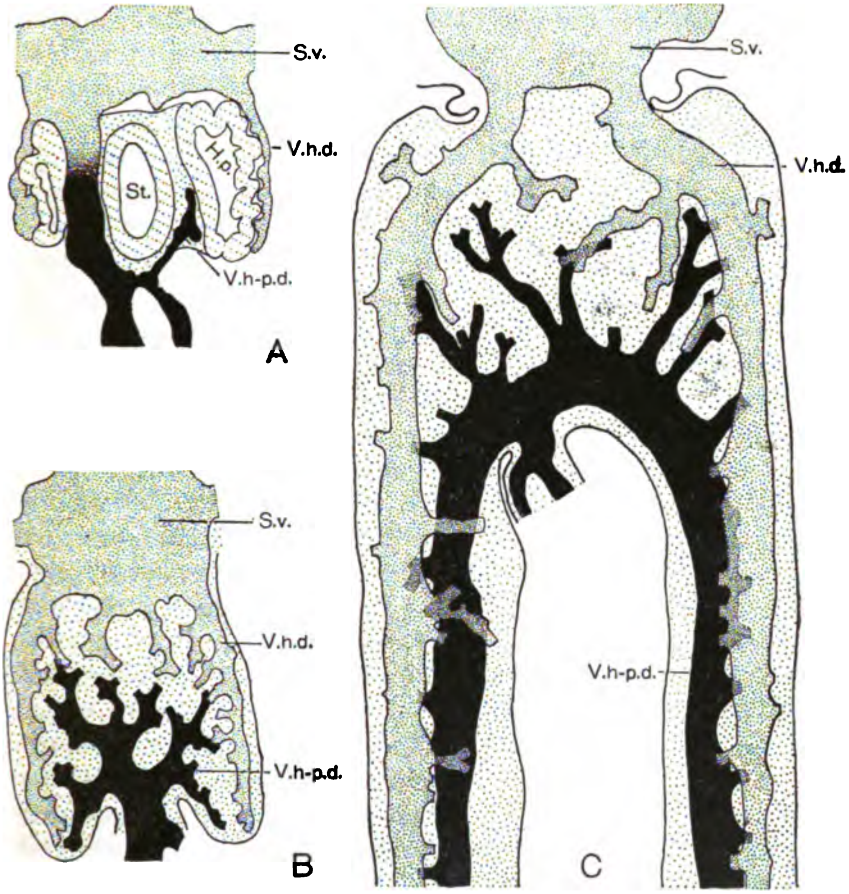

Fig. 11 Semi-diagrammatic graphic reconstructions (from frontal sections) of the liver veins, as seen from above, of three Acanthias embryos. The hepatic-portal veins are represented in solid black, the hepatic veins and sinus venosus in heavy stipple and the hepatic parenchyma in light stipple. In figure 12 A, the connection between the liver and the foregut and the upper parts of the lateral hepatic pouches are represented as cut away dorsally. *A*, embryo 15 mm. long (H.E.C. 230); *B*, embryo 20 mm. long (S.C. 31); *C*, embryo 41 mm. long (H.E.C. 371). *H.p.*, right lateral hepatic pouch; *St.*, stalk connecting liver pouch with foregut above; *S.v.*, sinus venosus; *V.h.d.*, right hepatic vein; *V.h.-p.d.*, right hepatic-portal vein.

along on the lateral surfaces of lateral hepatic pouches have formed a vascular plexus consisting of several large sinuses interrupted by small lacunae. In the drawing the omphalo-mesenteric veins, properly speaking, are represented in solid black and the sinus venosus and the veins developed from it in stipple. The posterior part of the omphalo-mesenteric veins may now be called 'hepatic-portal' veins, this being the term commonly used to designate them in the adult, while the veins which are efferent in their drainage will be designated by the term hepatic veins, which is the name applied to them in works on adult anatomy.

In the stage just described tubules have formed only on the lateral surfaces of the hepatic pouches. Soon thereafter tubule formation takes place with great rapidity along the margins of the pouches and from the pars hepatic medialis as well. The latter process results in breaking up the clear passageway of the blood along the left omphalo-mesenteric vein to the sinus venosus and in place of this one discrete passage there are formed a number of small irregular venous channels which pass from the posterior portions of the omphalo-mesenteric veins (hepatic-portal veins) in among the tubules and join the hepatic veins.

At this time there is a marked growth of the posterior lobes of the liver which brings about several changes in the position of the venous trunks. These may be seen by comparing figures 11 B and 11 C which are graphic reconstructions of embryos 18 mm. and 41 mm. long respectively. The branches of the early hepatic-portal vein which extend backward along the inner surface of the posterior lobes assume more and more importance, becoming in the later stage the posterior continuations of the main trunks of the vein. The former main trunks which extended forward are relatively reduced in size and appear as branches of these posterior vessels. At the same time the sinus venosus becomes more completely separated from the liver as the septum transversum is formed and the lateral hepatic efferent trunks converge towards the middle line. In so doing they take up the two larger venous radicles which formerly lay medial to them and represented the remains of the extreme anterior ends of the former omphalo-mesenteric veins. Thus these vessels, as may be seen by comparing *B* and *C* of figure 11, become branches of the hepatic veins instead of direct tributaries of the sinus venosus. In older embryos an increase in the caliber of the hepatic veins posterior to their entrance into the sinus venosus indicates the position of the hepatic sinuses which become so prominent in some selachians and which have been discussed in some detail by Neuville ('01).

In selachians as compared with the higher groups of vertebrates the primitive liver veins are retained in the adult almost in their entirety. The main vessels are established early before the sinusoidal complex is well developed and these trunks remain almost complete, although they may vary in size with changes in the hepatic parenchyma. In figure 14, for example, the main trunks of the hepatic vein can be seen in each section although the size of these trunks varies greatly. The only primitive hepatic vessel which is really completely broken up into sinusoids by the hepatic cylinders is the small segment of the left omphalomesenteric vein which passes over the pars hepatica medialis to join the sinus venosus and even the anterior and posterior ends of this vessel remain as branches of the left hepatic and the hepatic-portal veins respectively. The growth in length of the hepatic and hepatic-portal veins takes place at the ends of the lateral lobes of the liver. Here there exist venous sinuses which are joined by both veins and which are interrupted by only occasional hepatic cylinders sheathed with endothelium. From these sinuses the posterior ends of the afferent (hepatic-portal) and efferent (hepatic) veins are differentiated by the growth of a septum of hepatic cylinders which at first form a loose meshwork and later become a compact wall through which only small capillary sinusoids pass from one vein to another. Some of the venous branches of the second order represent remains of original venous trunks as for example the two largest tributaries of the hepatic veins from the median lobe. The others are the representatives of the larger passageways which have pushed between the fairly constant tubule clusters which arise from the hepatic pouches. Each of the larger branches of the hepatic-portal vein in the median lobe of the liver accompanies for a short distance a secondary hepatic duct derived from one of these tubule clusters. As a rule the tubule ridges of the lateral surfaces of the hepatic pouches are completely formed and are beginning to break up into rows of individual anlagen before these blood vessels invade their vicinity. Different embryos, however, show some variation in this respect. The vessels pass backward between these rows of tubules and form a vascular plexus interrupted by a few small lacunae. After this process is quite well under way, the main vascular trunk of the left omphalo-mesenteric vein is interrupted by the growth of the pars hepatica medialis and as a result vascular sprouts extend out from the posterior part of this vessel and its fellow of the opposite side. These sprouts join with the plexuses of the hepatic veins and there is thus produced a set of sinusoidal vessels connecting the remains of the omphalo-mesenteric (hepatic-portal) venous system with the true hepatic veins. The extension of this network is gradual, and is not completely established until the tubules are well differentiated and anastomosis between the tubules begins to take place. The establishment of the complete sinuosidal circulation and of tubule anastomosis is practically simultaneous. In Acanthias, as in the pig according to Hilton ('03) and in reptiles (Hammer '93), there is no reason to believe that the blood vessels have anything to do with the formation of the earliest hepatic tubules. Certainly there is no breaking up of a solid mass of liver cells as described in the frog by Shore ('93) or any indentation of the wall of the lateral liver pouches as has been seen in Torpedo by Choronshitzky ('00). The vessels last of all penetrate among the tubules of the ventral surface of lateral hepatic pouches. Figure 12 is a frontal section through the tubules of this region of an embryo 15 mm. in length (H.E.C. 228). It will be seen that the tubules are separated only by scattered mesenchymal cells with the exception of a single vascular sprig.

Minot ('00) has remarked that in the liver, and in the pronephros and mesenephros as well, the sinusoids which at first may be small, increase in size until they reach a certain maximum and then decrease. To secure some idea of the extent of the changes which take place in this process, the area in cross-section of the blood spaces of selected sections have been measured from a series of livers of selachian embryos. Eight sections were selected for measurement from each liver. To secure sections representing about the same relative planes in each specimen

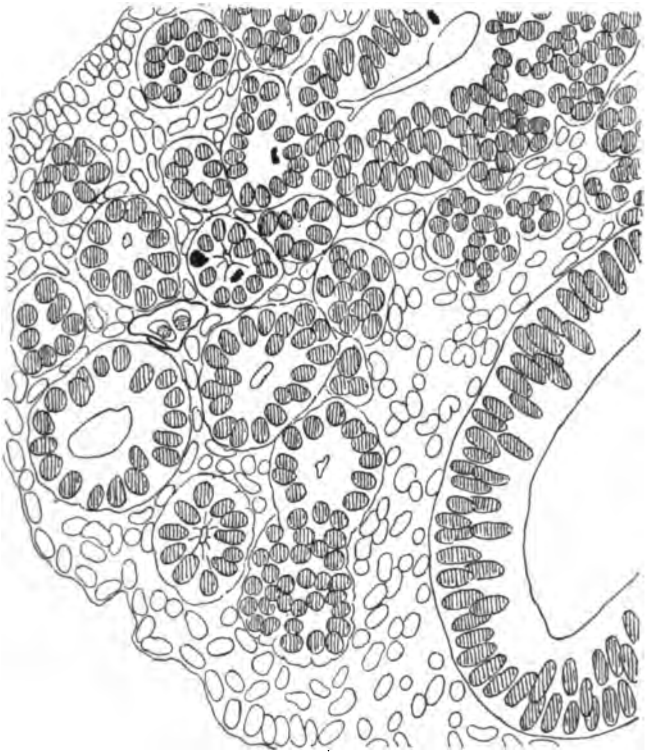

Fig. 12 Frontal section through the tubules arising from the ventral surface of the pars hepatica medialis of an Acanthias embryo 15 mm. long (H.E.C. 230). × 150. Hepatic cell nuclei are shaded with vertical lines, nuclei of mesenchymal cells drawn in outline. A single vascular twig is seen in cross section above the largest hepatic tubule.

the following method was employed. The liver was considered as divisible into three parts, as represented in figure 13. The first segment (*A* to *B*, fig. 13) consisted of that part of the liver anterior to the foremost part of the curved cystic duct. The second segment (*B* to *C*) extended from the cystic duct to the plane where the anterior lobe of the liver joins with the two lateral and posterior ones. The two lateral lobes form the third segment (*C* to *D*). The first segment, *A* to *B*, was divided into three equal parts and the sections falling upon the planes separating these parts (*1* and *2*) were used for measurement. Two sections were selected in the same manner from the second segment *B* to *C*. The third segment *C* to *D* was divided into five equal parts and the four sections falling on the dividing planes used for measurement. In this segment only the left lobe was measured in each case. The sections thus determined were drawn with the camera lucida or projection apparatus and the total area, the total area of the vascular bed in cross-section, and the area of the larger vascular trunks in cross-section were then determined by means of a planometer.[6] From this data the figures given in table 2 were calculated. Such a method can lay no claim to great accuracy. The possibility of error is quite large and the method of determining the position of the sections has some objections, for all parts of the liver do not grow at the

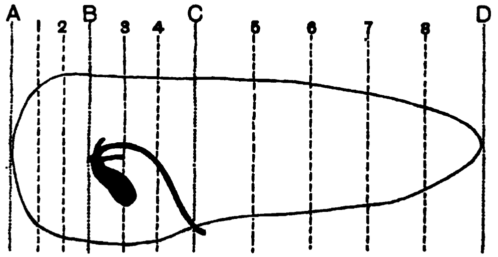

Fig. 13 Diagram of the liver of an Acanthias embryo showing method of selecting sections for measurements of the blood vessels. Bile ducts and gall bladder represented in black.

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[6] It is impossible to determine the exact area of the large trunks in the earlier stages because of their great irregularity and their many connections with the adjoining sinusoids. In embryos 20 to 21 mm. long the area of the main venous trunks forms about 20 per cent of the entire cross section area of the vascular spaces of the liver. This drops to about 15 per cent in embryos of 25 to 28 mm. and increases thereafter with the relative reduction of the sinusoids. In the last member of the series in table 2, the main trunks formed about 30 per cent of the total cross section area of the blood vessels.

same rate and the development of the vascular supply of the organ is closely related to its growth. Such a study, however, does give us some rough approximations which may be of value.

A study of table 2 brings out clearly the three stages in the vascularization of the liver in Acanthias. The first stage found in an embryo between 12 and 20 mm. in length is one in which the tubules are growing with great rapidity and the sinusoids are only cleft like endothelium lined spaces (figs. 14 A and B). It is only in the lateral lobes of the liver where the hepatic and the hepatic-portal veins unite in large sinuses that the vascular percentage of the organ is high. During the latter part of this period tubule anastomoses are forming in large number. With anastomosis the vascular supply of the organ increases with the greatest rapidity and the tubules, taking on the form of slender cylinders, are separated by very large vascular spaces. This phase, which is inaugurated rather suddenly, continues while the embryo grows from a length of about 20 mm. to a length of

TABLE 2

Measurements of the hepatic vascular spaces in Acanthias embryos

LENGTH OF EMBRYO IN MM.	DESIGNATION	AREAS IN MM.	LEVELS OF SECTION							
			1	2	3	4	5	6	7	8
15.0	H.E.C. 227	T.a.[1] V.a.[1] V.%[1]	0.238 0.035 **18.3**	0.321 0.044 **13.8**	0.314 0.020 **6.3**	0.314 0.021 **6.7**		0.029 0.005 **26.3**		0.019+ 0.006− **30.0**
20.5	S.C. 5	T.a. V.a. V.%	0.308 0.030 **9.8**	0.423 0.047 **11.2**	0.581 0.031 **14.0**	0.502 0.112 **22.4**	0.160 0.172 **10.8**	0.099 0.024 **24.5**	0.065 0.012 **19.7**	0.027 0.007 **27.7**
20.6	H.E.C. 1494	T.a. V.a. V.%	0.738 0.349 **47.3**	0.942 0.373 **39.7**	0.742 0.385 **52.0**	0.934 0.392 **42.0**	0.412 0.172 **41.8**	0.232 0.124 **53.4**	0.234 0.141 **60.3**	0.178 0.112 **68.5**
28.0	S.C. 6	T.a. V.a. V.%	1.120 0.698 **62.4**	1.440 0.616 **42.8**	1.210 0.591 **48.9**	1.190 0.522 **43.9**	0.357 0.205 **57.5**	0.324 0.167 **51.5**	0.303 0.173 **57.0**	0.204 0.157 **77.0**
36.6	S.C. 29	T.a. V.a. V.%	2.060 0.403 **19.6**	2.120 0.578 **27.3**	2.340 0.559 **23.9**	2.270 0.644 **28.4**	0.669 0.152 **22.8**	0.595 0.108 **18.3**	0.590 0.084 **14.4**	0.357 0.051 **14.4**

[1] T.a., total area of cross section in square mm.; V.a., area of vascular spaces in square mm.; V.%, percentage of total area occupied by vascular spaces.

about 30 mm. During this period roughly one-half of the bulk of the liver is made up of vascular spaces and of this half much the greater part is in the form of sinusoids. Figure 14 C is a section through a plane corresponding to figure 14 B, and shows graphically the extent of the blood vessels at the time when they form the larger part of the liver. New tubules develop in great numbers during this time. Finally a period of reduction in the size of the sinusoids sets in. This reduction is brought about entirely by the increase in the size of tubules already formed. By this increase in the parenchyma the sinusoids are reduced to the 'capillary sinusoids' of Minot. This process is very noticeable in embryos between 35 and 45 mm. in length. It continues probably to the time of birth. Figure 14 E is of a cross section of the liver of an embryo 47.3 mm. long and shows the marked decrease in the size of the sinusoids at that time.

The increase in size of the sinusoids is both actual and relative. The decrease in the total area (in cross-section) of the sinusoids is at first only a relative one, but later, for a short period at least, it is actual as well as relative.

A point which table 2 does not bring out is that the size of the sinusoids is not determined by their position in relation to the larger vessels but is dependent upon the stage of development of the parenchyma with which they are interwoven. The sinusoids do not form a tapering system of vessels largest near the veins which receive them. This has been in a way pointed out in the section upon the growth of the hepatic cylinder network. In the lateral lobes for example, the growth of the tissue is at first entirely backward and during this period sinusoids of large size are generally distributed throughout the lobe (fig. 15 A). After the lobes have completed the greater part of their posterior growth there begins a great increase in the hepatic tissue along their dorsal margins and the lobes gradually push upward on either side of the intestine. With this change in the area of rapid growth there also occurs a change in the sinusoids which, as is shown in figure 15 B, continue to form practically half of the dorsal portion of the lobes while in the ventral portions they form less than 10 per cent of the total area in cross-section.

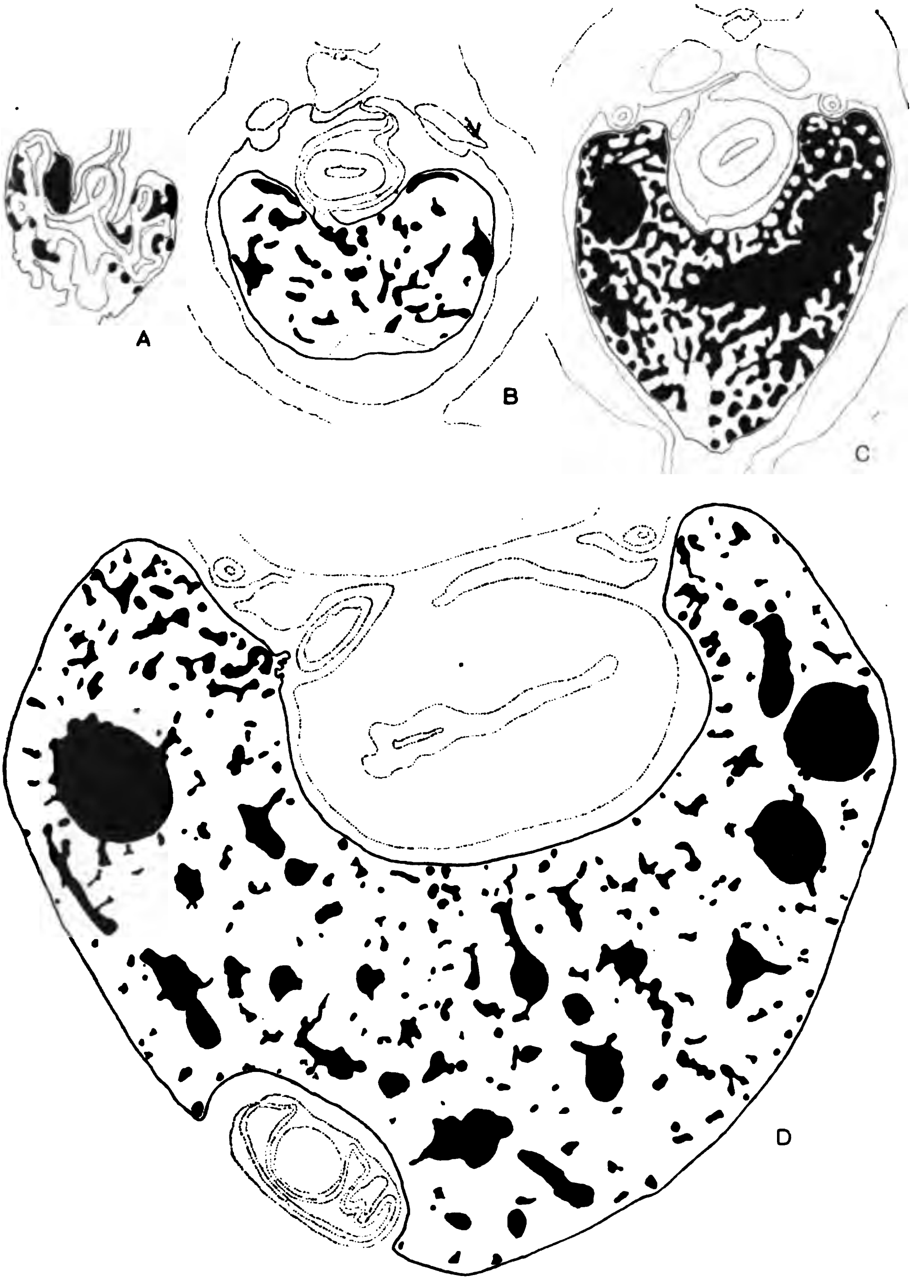
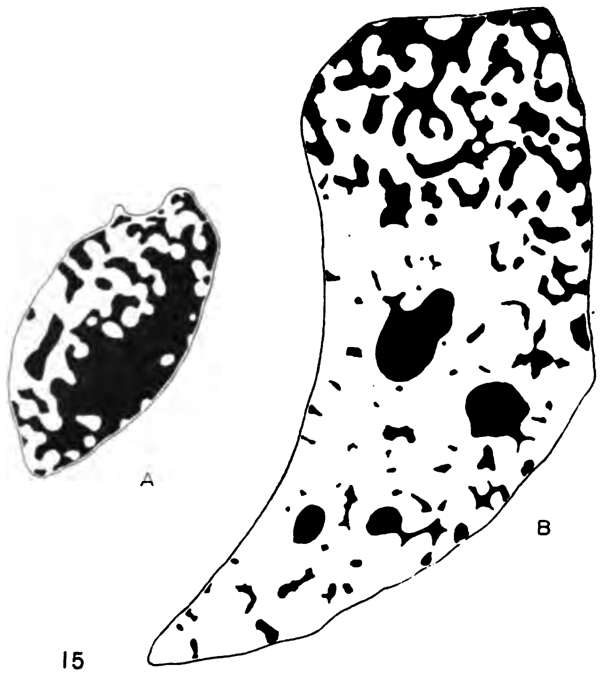


Fig. 14 A series of transverse sections of the livers of Acanthias embryos of different ages showing the development of the sinusoids which are represented in solid black. All sections, with the exception of *A*, are taken at a plane equally distant from the anterior end of the liver and the anterior end of the gall bladder; all × 35. *A*, embryo 13.3 mm. long (S.C. 18); *B*, embryo 20.5 mm. long (S.C. 5); *C*, embryo 28 mm. long (S.C. 6); *D*, embryo 47.3 mm. long (S.C. 11).

Similar changes are seen in earlier stages in the main median lobe of the liver and in the process of the cystic lobe which extends into the yolk stalk coelome.

In Mustelus and Torpedo the early development of the hepatic sinusoids is quite different from that seen in Acanthias. In these forms the omphalo-mesenteric veins become much enlarged at an early stage and lie both dorso-medial and ventro-lateral to the hepatic pouches instead of only medial to them in Acanthias. As a result the hepatic tubules are in contact with the walls of large vascular chambers from the time of their first appearance and as they grow they can only do so by pushing the endothelial walls of the vessels before them. The tubules, as has been remarked, are slender and elongated and unite by anastomoses soon after their formation. Thus there is no stage in these forms corresponding to the early one in Acanthias where the sinusoids are small cleft-like spaces. The proportion of the volume of the liver formed by sinusoids is very high at first but is steadily reduced as development proceeds. Figure 16 of two transverse sections of Mustelus embryos 16.7 mm. and 22.5 mm. in length respectively illustrates these points, as do also the thick frontal sections shown in figures 44 and 45. The larger vasculartrunks of the liver in Mustelus and Torpedo are mapped out by the growth of the hepatic cylinder network between them. As in Acanthias the main liver veins are marked out early in the development of the organ, and are not formed by the fusion of smaller vascular spaces.

In both the forms under discussion and in Acanthias the hepatic sinusoids are formed by the intercresence of the hepatic cylinders and the omphalo-mesenteric veins. In Mustelus and Torpedo this intercresence is due to the invasion of the space occupied by the vein. In Acanthias, *in early stages,* the intercresence is due to the penetration of the venous sprouts about the tubules which are already established. In later stages in Acanthias the cylinders increase by growing into venous spaces and the end result in either case is the same. Thus both the methods of sinusoid development postulated by Minot ('00) and by Lewis ('04) are found in these forms.



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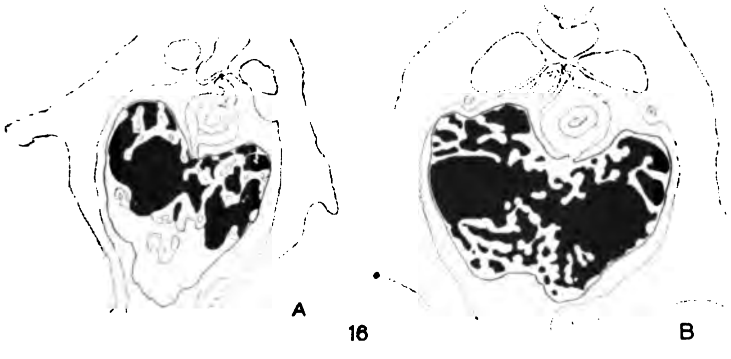

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Fig. 15 Two transverse sections through the middle of the left lateral lobe of Acanthias embryos; both × 75. *A*, embryo 28 mm. long (S.C. 6); *B*, embryo 47.3 mm. long (S.C. 11); sinusoids represented in black.

Fig. 16 Transverse sections through the middle of the median lobe of two embryos of Mustelus canis. *A*, embryo 16.7 mm. long (S.C. 32); *B*, embryo 22.5 mm. long (S.C. 33); both × 35; sinusoids represented in black.

In Acanthias the establishment of the sinusoidal circulation and the process of anastomosis of the hepatic tubules occur almost simultaneously. In Mustelus and Torpedo the hepatic tubules anastomose immediately after their intercresence with the omphalo-mesenteric veins. At first sight there would seem to be some relation between the sinusoidal type of circulation and the anastomosis of glandular end-pieces. This seems to be supported by the evidence that the pancreas of ganoids has free anastomoses with a sinusoidal circulation and these conditions are also found in the paraphysis of Necturus (Warren '05) and in the islands of Langerhans in Mammalia (deWitt '06). Sinusoids also occur in other ductless glands which anastomose. On the other hand I have found no report of anastomoses between the tubules of the mesonephros, and a free sinousoidal circulation exists in this organ. The tubules of the embryonic mammalian pancreas are known to anastomose freely, and there is no record of a sinusoidal circulation in this organ. Similarly Braus ('00) finds a free anastomosis of the end pieces of the bulbo-urethral gland which has the capillary type of circulation and Bremer ('11) has found the same conditions in the testis.[7] It is evident that no general rule can be laid down regarding this relation, at least with our present knowledge of the subject.

SUMMARY

A. Development of the hepatic parenchyma

1. Hepatic tubules are first represented by longitudinal ridges formed on the external surfaces of the pars hepatica lateralis and medialis, and by slight irregularities of the margins of the pouches forming the pars hepatica lateralis.
2. The hepatic ridges are converted into irregular rows of tubule anlagen by transverse constructions.
3. The individual tubule anlagen thus formed grow outward and are differentiated into expanded terminal chambers and

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[7] Occasional anastomoses are found in a number of glands such as the gastric and uterine glands. It is to be expected that such occasional connections will be found in almost any gland which is studied carefully with the aid of reconstructions.

constricted proximal necks. Secondary tubules of the second, third or fourth order may be produced from the terminal expanded chamber before tubule anastomoses are formed.

4. In Acanthias the hepatic tubules may differentiate in regions into which blood bessels have not penetrated.

5. Tubule anastomosis and the complete establishment of sinusoidal circulation are practically simultaneous.

6. Anastomoses of tubules may take place end to end, side to end, or side to side. The frequency of the several types is in the order stated.

7. The process of anastomosis involves the stages of (a) the contact and fusion of the tubules involved, (b) the rearrangement of cells in the area of confluence, and (c) the establishment of a new connecting lumen.

8. While the cell position is shifted in anastomosis, the cell axis remains unchanged.

9. After anastomosis the increase in the number of hepatic cylinders takes place in three ways, viz: (a) the formation of hepatic cylinders directly from the pouch wall; (b) the interstitial development of cylinders from blind sprouts from the network; (c) the peripheral growth of the terminal hepatic cylinders.

10. These methods of increase cease in the order named and contribute to the network inversely to the order given above.

11. Increase in the number of cylinders of the hepatic network first ceases in the body of the median lobe and last in the tips of the lateral lobes and along the dorsal margins of the lateral lobes and connecting portion.

12. The number of cells surrounding the lumen of the hepatic cylinder in cross-section drops from an average of 12 + in the tubule at the time of anastomosis (14 to 15 mm.) to 3 ± in an embryo 95 mm. long.

13. The diameter of the hepatic cylinders increases up to and immediately after the time of anastomosis. With the enormous increase in the size of the sinusoids following a short time after anastomosis, the diameter for a time decreases. Thereafter the diameter of the cylinders undergoes a steady increase at least to the time of birth or hatching.

14. The early increase in size of the cylinders is due to a multiplication of cells. The later increase is due to a growth in the size of the individual cells.

15 The hepatic nuclei which are originally large and elongately oval, become somewhat reduced in size and spherical in shape at the time when the tubules are formed from the tubule ridges. Thereafter they undergo a slow shrinkage in size up to the time of birth. In later stages they may again become oval or gibbus in outline, due to the pressure of the intracellular fat. The nuclei are at first basal in position and later become either central or peripheral.

16. Hepatic nuclei first have the common embryonic type of structure which they retain longer than do the nuclei of most tissue. The embryonic type of structure is lost first in the nuclei of the gall bladder and major hepatic ducts, next in the minor hepatic ducts, and finally in the hepatic cylinders.

17. Fat appears in the cylinders at an early stage and eventually fills almost the entire cell as in the typical fat cell.

18. Selachians in which the omphalo-mesenteric veins are early developed to large proportions have slender hepatic tubules which indent the walls of the vessels and anastomose at a very early stage.

B. *Development of the minor ducts*

1. Two forms of bile duct formation exist in the selachian liver, that of evagination of the liver pouch, and that of transformation of pre-existing cylinders into bile ducts. The first and most primitive is more active in the selachians than in higher vertebrates and forms the major ducts and the proximal parts of the minor ones. The distal parts of the minor ducts are formed by cylinder transformation.

2. Ducts are only differentiated from cylinders which lie in contact with branches of the omphalo-mesenteric veins. The side of the cylinder lying towards the vein is always the first to differentiate and in the smaller ducts is the only part to be differentiated from the true hepatic cells.

3. The cells of the bile duct epithelium are structurally farther removed from the primitive hepatic cell type than are the cells of the hepatic cylinders.

C. The hepatic mesenchyma

1. The hepatic mesenchyma is derived from the splanchnic mesothelium from two zones and at two periods. In both cases the mesenchymal proliferation is associated with marked irregularities of the mesothelium.

2. The first mesenchymal proliferation is ventral and is associated with the formation of mesothelial villi on the right side and irregular mesothelial growths about the omphalo-mesenteric vein on the left side. This proliferation forms the mesenchymal tissue about the gall bladder, cystic duct, main trunk of the omphalo-mesenteric vein, and to an indeterminable degree the interstitial mesenchymal tissue of the ventral part of the liver.

3. The second and dorsal proliferation is associated with the formation of mesothelial funnels and anastomosing mesothelial tubules which at one time occupy the dorsal fifth of the liver. These mesothelial invaginations break down into mesenchymal cords which in turn form the interstitial mesenchymal tissue of the dorsal part of the liver.

4. These two zones of mesenchymal proliferation correspond with the two zones of splanchnic mesenchymal proliferation of the trunk region of selachian embryos as described by H. E. Ziegler.

5. The mesothelial covering of the liver is at first made of columnar cells which later become squamous and thereafter apparently give rise to little or no mesenchymal tissue. The last areas from which the primitive form of mesothelium disappears are the tips of the lateral lobes and the dorsal margins of the lateral and median lobes.

6. Generally the splanchnic mesothelium becomes reduced at once from a columnar to a squamous cell layer upon contact with a hepatic tubule or cylinder.

7. The primitive embryonic type of nuclear structure is lost in the mesenchymal cells as they are proliferated from the mesothelium. No sharp line of demarcation could be drawn between endothelial and mesenchymal cells.

D. Hepatic sinusoids

1. Hepatic sinusoids arise in selachians by intercresence of the hepatic cylinders with the omphalo-mesenteric vein. This intercresence may be brought about either (a) by the growth of the rami of the omphalo-mesenteric vein about the cylinders as in Acanthias, or (b) by the invagination of the vessel wall by growing cylinders as in Mustelus and Torpedo.

2. In the first type there may be recognized three stages of sinusoid development: (a) The first in which the sinusoids are sparse and differ from capillaries only in their terminations at either side in veins; (b) The second stage in which the sinusoids are greatly enlarged constituting approximately 50 per cent of the liver; (c) The final stage in which the sinusoids take the form of the capillary sinusoids of Minot. In the second type of intercresence only the last two stages are present.

3. The reduction of the sinusoids from stage (b) to stage (c) is due to the growth in size of the hepatic cylinders and not due to their increase in number, for the most active increase in number of cylinders takes place prior to or early in stage (b).

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PLATE 1

EXPLANATION OF FIGURES

17 Reconstruction of the hepatic anlage of an Acanthias embryo 7.5 mm. long (S.C. 14) showing longitudinal tubule ridges upon the wall of the lateral hepatic pouch.

18 Reconstruction of the hepatic anlage of an Acanthias embryo 10 mm. long (S.C. 20) showing the formation of individual tubule anlagen from the tubule ridges.

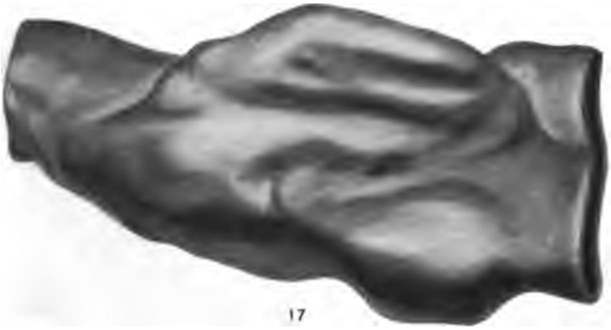
19 Reconstruction of a hepatic tubule of an Acanthias embryo 13.3 mm. long (S.C. 18) showing division into dorsal chamber and proximal neck and the secondary tubules arising from the former.

20 Reconstruction of a more highly developed tubule; from the same specimen as figure 19.

21 Reconstruction of two simple tubules from the lateral hepatic pouch of an Acanthias embryo 15 mm. long (H.E.C. 227).

22 and 23 Two views of a reconstruction of a highly developed hepatic tubule just prior to anastomosis; some of the secondary tubules are in contact; from the same specimen as figure 21.

24 Reconstruction of three anastomosing tubules; from the same specimen as figure 21; tubules *A* and *B* are completely fused; tubules *C* and *A* are in an early stages of the process.



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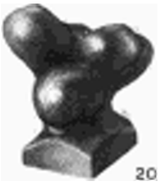

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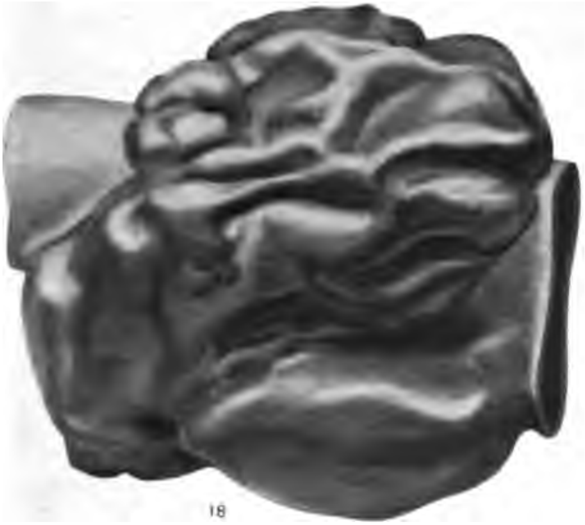

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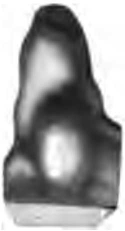

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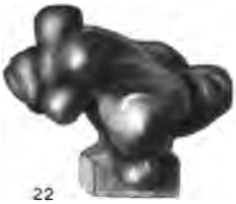

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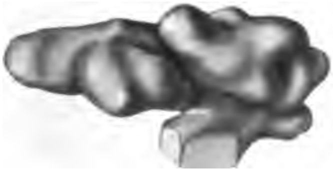

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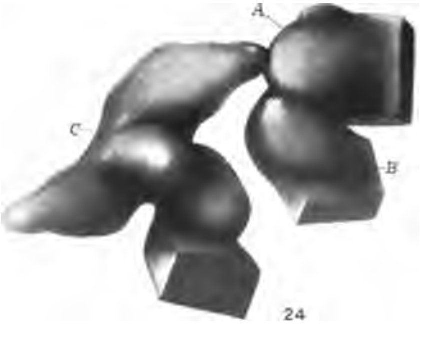

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PLATE 2

EXPLANATION OF FIGURES

25 to 27 Sections of hepatic tubules of Acanthias illustrating the process of anastomosis; (fig. 36 represents a step between figs. 26 and 27).

25 Early contact stage in anastomosis; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.

26 Fusion of tubules; from an embryo 19 mm. long (S.C. 3); alum hematoxylin. × 400.

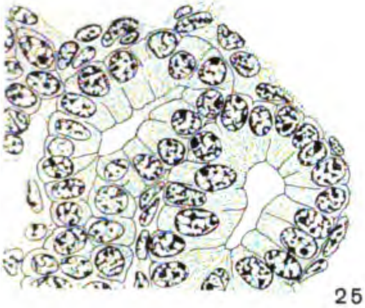
27 Establishment of connecting lumen between two anastomosed tubules. From an embryo 19 mm. long (S.C. 2); alum hematoxylin. × 400. Graphic reconstructions of these tubules, shown in cross section in figures 26 and 27, are illustrated in figure 2, page 224.

28 to 30 Sections of the external walls of lateral hepatic pouches of Acanthias embryos illustrating the formation of the hepatic tubules.

28 Formation of tubule ridges; from an embryo 8 mm. long (K.U.E.C. 545); iron hematoxylin. × 600.

29 Early outpouching of individual tubules. From an embryo 10 mm. long (S.C. 20); alum hematoxylin. × 400.

30 Completely formed tubules at the time when anastomoses first appear; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.



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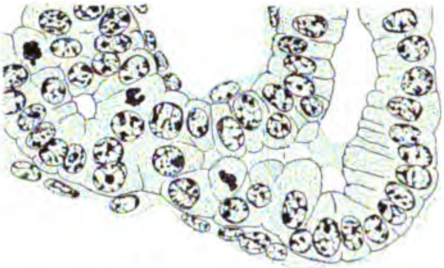

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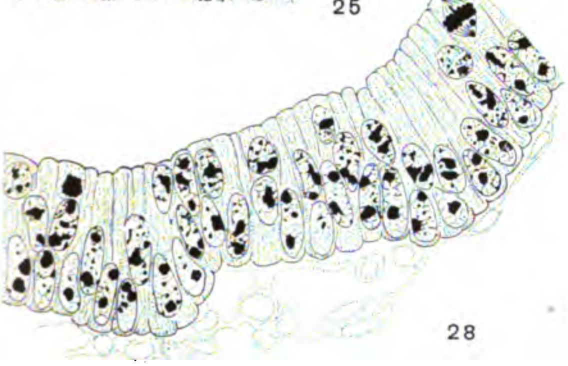

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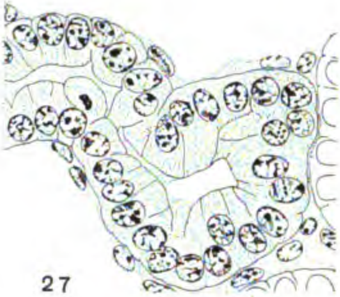

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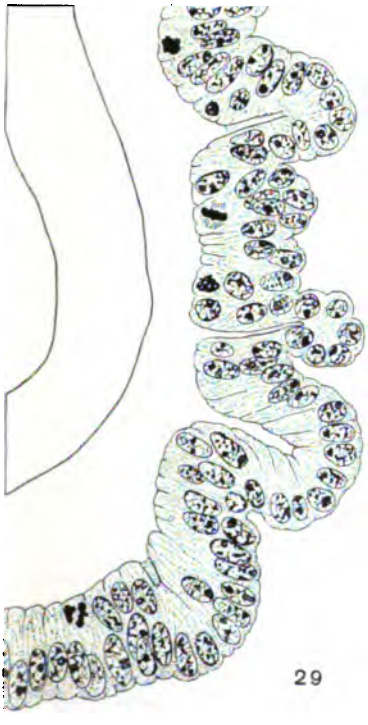

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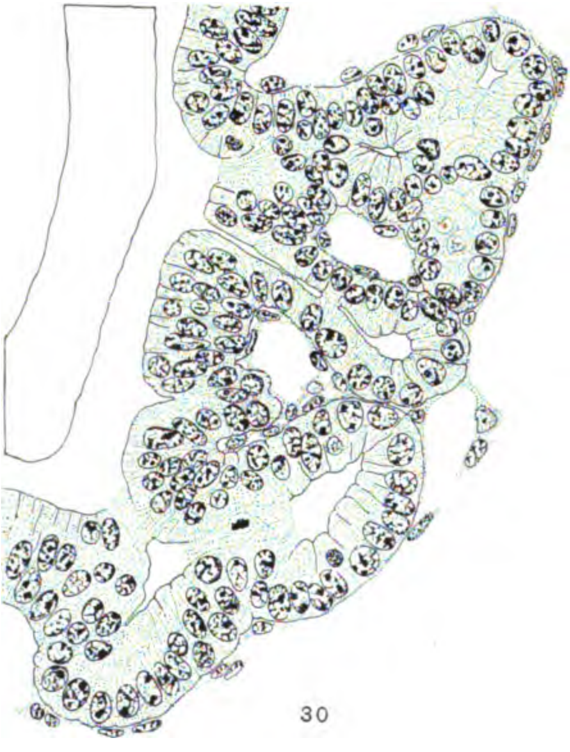

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PLATE 3

EXPLANATION OF FIGURES

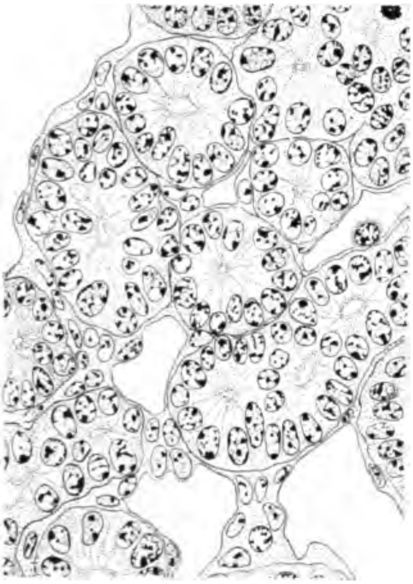
31 to 34 Four sections of livers of Acanthias embryos of different ages, illustrating the changes in the hepatic cylinders; all × 400.

31 Section of the liver of an embryo 16 mm. long (K.U.E.C. 547); iron hematoxylin.

32 Section of the liver of an embryo 20.6 mm. long (H.E.C. 1494); iron hematoxylin.

33 Section of the liver of an embryo 32 mm. long (H.E.C. 1652); iron hematoxylin.

34 Section of the liver of an embryo (of Squalus sucklii) 95 mm. long (H.E.C. 1882); alum cochineal.



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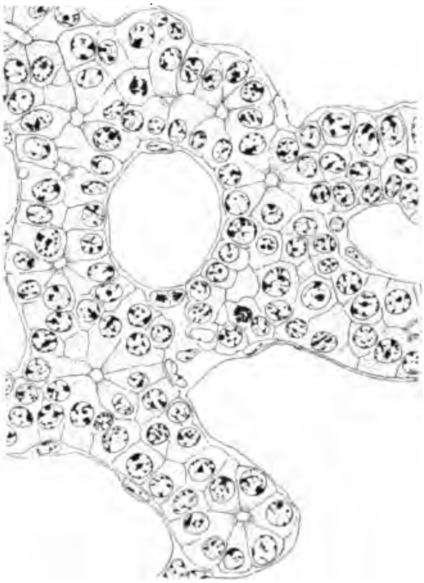

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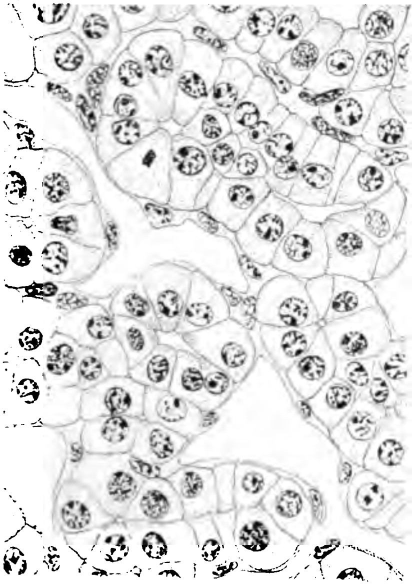

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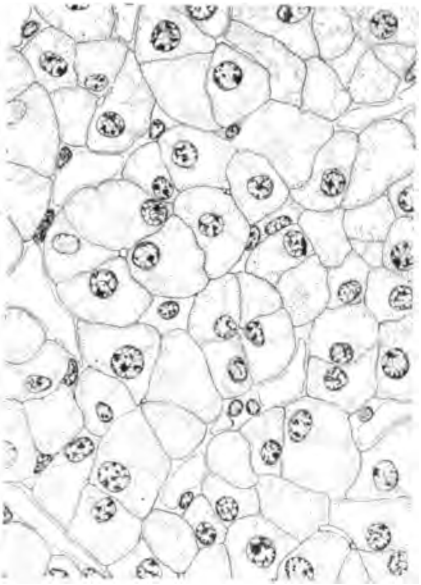

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PLATE 4

EXPLANATION OF FIGURES

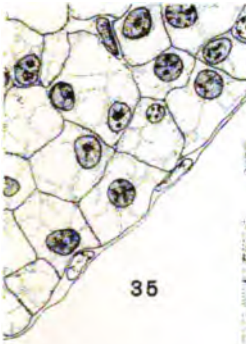
35 Transverse section of the margin of the liver of an embryo of Torpedo ocellata 29 mm. long; alum hematoxylin. × 350.

36 Section of an anastomosis between two hepatic tubules of an Acanthias embryo 14 mm. long (S.C. 30); alum hematoxylin. × 500. (This section shows a stage intermediate to those shown in figures 26 and 27).

37 Transverse section of a terminal bile duct of an embryo 95 mm. long (H.E. C. 1882); alum cochineal. × 400.

38 Hepatic tubules and mesenchymal ingrowths from a transverse section of the dorsal margin of the liver of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400. *M*, mesenchymal ingrowths. *H.t.*, hepatic tubules.

39 Transverse section of a developing bile duct of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400.



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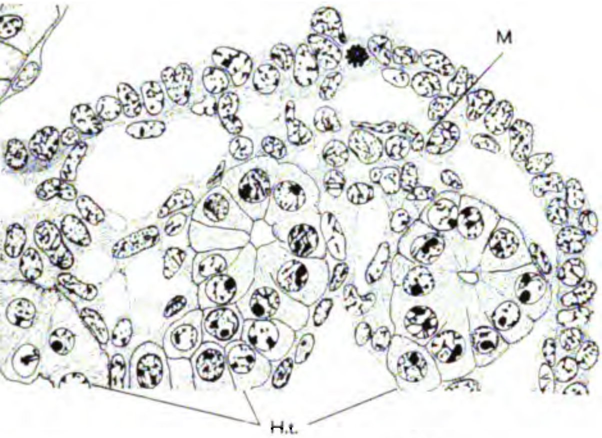

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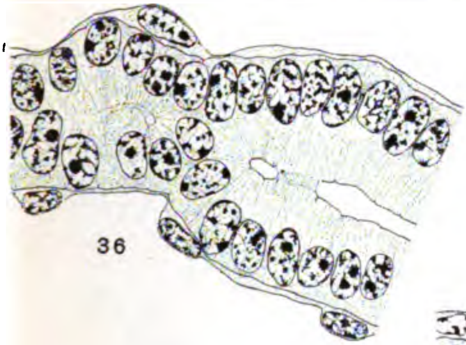

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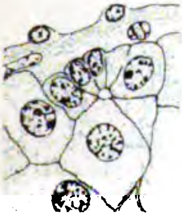

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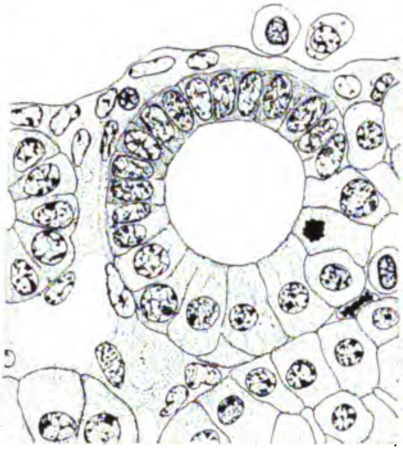

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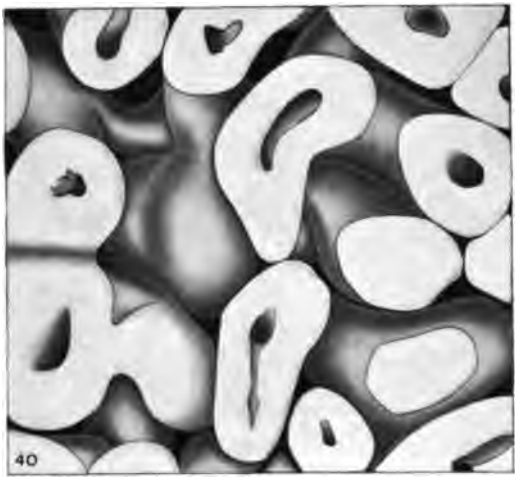
PLATE 5

EXPLANATION OF FIGURES

40 to 42 Portions of thick sections of livers of Acanthias embryos of different ages. The method of preparation is described in footnote 4, page 225. All approximately × 200.

40 Thick section of the liver of an embryo approximately 15 mm. long.
41 Thick section of the liver of an embryo approximately 21 mm. long.
42 Thick section of the liver of an embryo approximately 30 mm. long.



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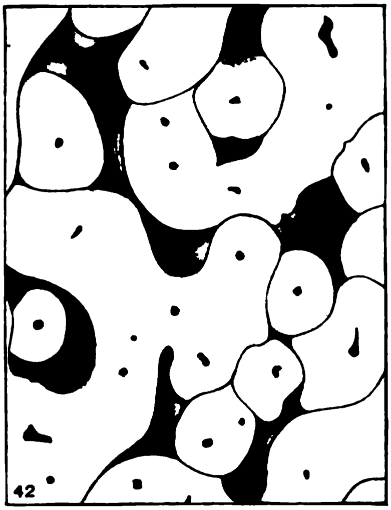

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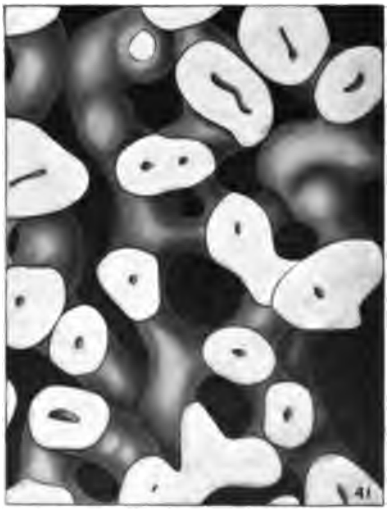

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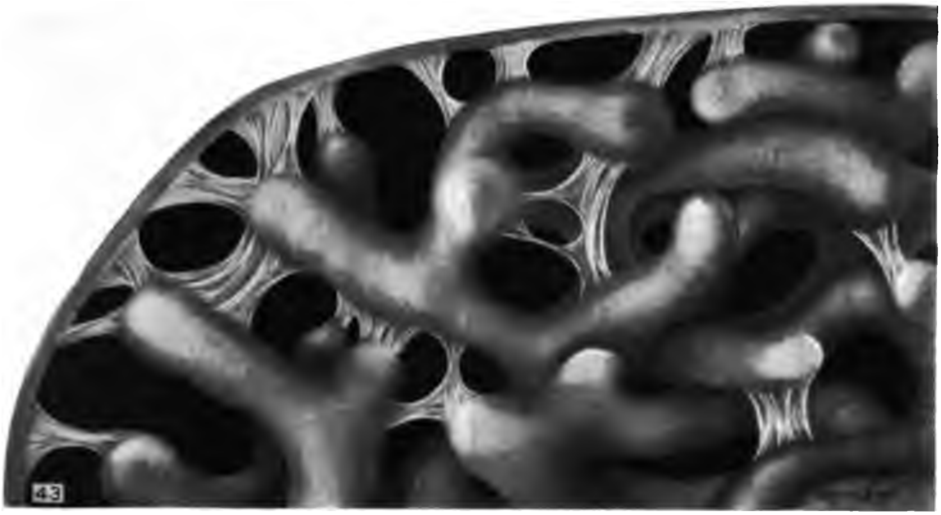
PLATE 6

EXPLANATION OF FIGURES

43 Preparation of the periphery of the posterior lobe of an Acanthias embryo approximately 20 mm. long showing the terminal buds of the hepatic network and the attachment of the network to the mesothelial covering by means of mesenchymal strands.

44 Thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The large hepatic duct and the hepatic tubules lie in a vascular sinus which is represented in black. The vertical band seen on the right hand side is a section of the mesothelial covering of the liver.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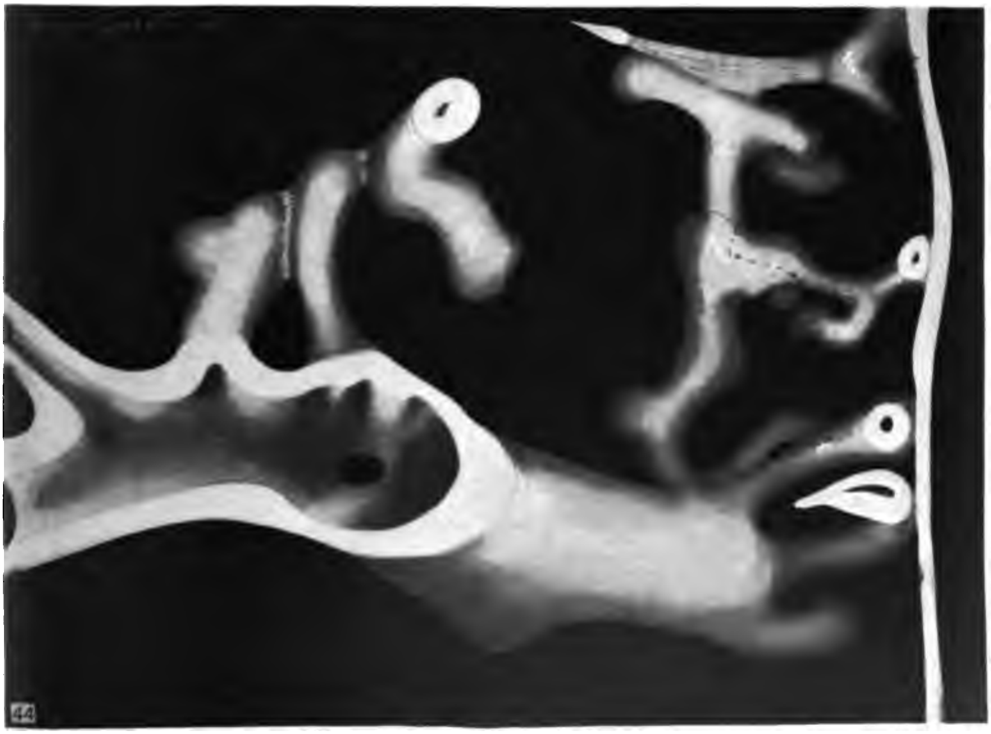
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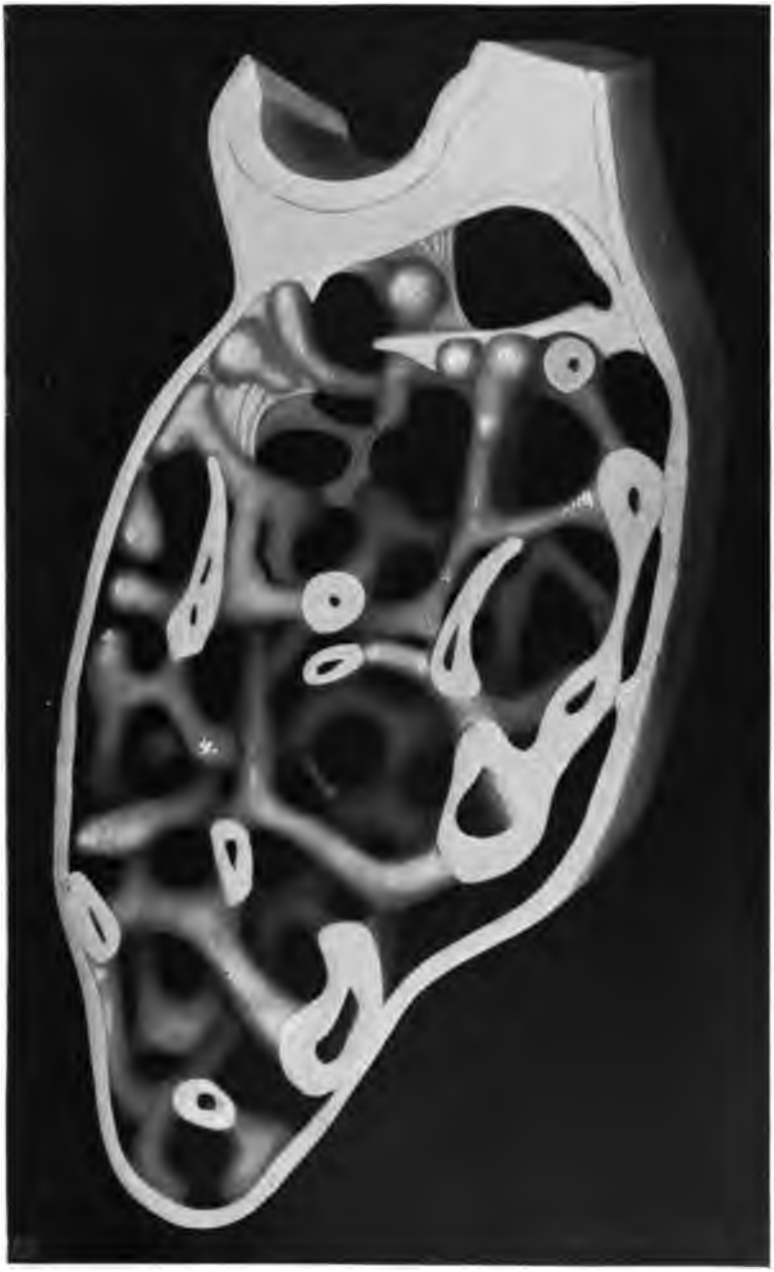
PLATE 7

EXPLANATION OF FIGURE

45 Thick frontal section of the liver of an embryo of Mustelus canis approximately 16 mm. long.



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# CONTRIBUTIONS
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VOLUME 4

1916

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CONTRIBUTIONS
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CONTENTS

The following contributions have been published during the year 1916. Papers marked with an asterisk (*) are not included.

Allen, William F. 1. Studies on the spinal cord and medulla of Cyclostomes with special reference to the formation and expansion of the roof plate and the flattening of the spinal cord. Journal of Comparative Neurology, 26: 9-77, 87 figures.

Baumgartner, E. A. 1. The development of the liver and pancreas in Amblystoma punctatum. American Journal of Anatomy, 19: 211-275, 46 figures, 4 plates.

Hoskins, E. R. 1. The growth of the body and organs of the albino rat as affected by feeding various ductless glands (thyroid, thymus, hypophysis, and pineal). Journal of Experimental Zoology, 21: 295-346, 4 charts.

Jackson, C. M. 1. Effects of inanition upon the structure of the thyroid and parathyroid glands of the albino rat. American Journal of Anatomy, 19: 305-352, 14 figures.

2. Embryology and medicine. Journal-Lancet, May 15.

*3. Effects of inanition upon the structure of the thyroid and parathyroid glands of the albino rat. (Abstract.) Proceedings American Association of Anatomists, Anatomical Record, 10, (Jan.).

Johnston, J. B. 1. Evidence of a motor pallium in the forebrain of reptiles. Journal of Comparative Neurology, 26: 475-479, 1 figure.

2. The development of the dorsal ventricular ridge in turtles. Journal of Comparative Neurology, 26: 481-503, 27 figures.

3. Neutral red as a cell stain for the central nervous system. Anatomical Record, 11: 297-298.

Myers, J. A. 1. Studies on the mammary gland. I. The growth and distribution of the milk-ducts and the development of the nipple in the albino rat from birth to ten weeks of age. American Journal of Anatomy, 19: 353-389, 6 figures, 4 plates.

*2. Growth and distribution of the milk-ducts and development of the nipple in the albino rat from birth to ten weeks of age. (Abstract.) Proceedings American Association of Anatomists, Anatomical Record, 10 (Jan.).

Norris, Edgar H. 1. The morphogenesis of the follicles in the human thyroid gland. American Journal of Anatomy, 20: 411-448, 17 figures.

Scammon, Richard E. 1. On the development of the biliary system in animals lacking a gall-bladder in postnatal life. Anatomical Record, 10: 543-558, 10 figures.

*2. The development of the biliary system in animals lacking a gall-bladder in post-embryonic life. (Abstract.) Proceedings American Association of Anatomists, Anatomical Record, 10 (Jan.).

Stewart, Chester A. 1. Growth of the body and of the various organs of young albino rats after inanition for various periods. Biological Bulletin, 31: 16-51, 5 tables, 4 charts.

*2. Growth of the body and of the various organs of young albino rats upon refeeding after inanition for various periods. (Abstract.) Proceedings American Association of Anatomists, Anatomical Record, 10 (Jan.).

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February, 1916

STUDIES ON THE SPINAL CORD AND MEDULLA OF CYCLOSTOMES WITH SPECIAL REFERENCE TO THE FORMATION AND EXPANSION OF THE ROOF PLATE AND THE FLATTENING OF THE SPINAL CORD[1]

WILLIAM F. ALLEN

Department of Anatomy, University of Minnesota

EIGHTY-SEVEN FIGURES

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INTRODUCTION

This paper has grown out of the study of the caudal heart and the spinal cord and nerves related to it in Polistotrema. Parts of that study which are not yet completed will be published later and will deal with the origin, distribution, and phylogeny of the spinal nerves; the origin of muscle sense organs in connection with the specialized muscles of the caudal heart; and certain ganglion cells, possibly sympathetic, which are found along the course of the spinal and vagus nerves. The present paper includes a study of the mode of origin of the fourth ventricle in several groups, an account of a structure similar to the fourth ventricle found in the caudal part of the spinal cord of an adult Polistotrema, and a study of the development of the central canal, and the causes underlying the flattening and the ventral indenting of the spinal cord in Cyclostomes.

---

[1] A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Department of Anatomy, The University of Minnesota.

Material and its preparation

Since the material for this paper came from such diverse sources, and such different modes of technique were employed, too much time and space would be consumed if a detailed description were given of each method employed. With the exception of the Petromyzon material, which was fixed in Flemming, corrosive-acetic, and Perenyi's fluid, my own material was fixed either in Tellyesnicky's or Bouin's fluid. It was sectioned after paraffin or the combined celloidin-paraffin method of imbedding. The latter method gave by far the better results, since it appears to have all of the advantages of celloidin in causing almost no shrinkage and its ability to hold yolk granules and blood corpuscles intact; besides allowing the sections to be cut as thin as paraffin alone, and causing no more difficulty in manipulation. For the most part the sections were stained in Heidenhain's iron hæmatoxylin and counterstained in an alcoholic solution of orange G plus a little acid fuchsin. In a few instances, in very young Petromyzon embryos, where all the tissues were filled with yolk, carmine and hæmalum were used to advantage.

Acknowledgments are due to Prof. T. G. Lee for the gift of a very complete series of Petromyzon embryos, which were obtained from the Connecticut River. Also to Prof. R. E. Scammon for the loan of his very complete serial collection of Squalus embryos, and to Prof. J. B. Johnston for the use of a similar serial collection of Amblystoma embryos. The splendid series of human and pig embryos belonging to the Institute of

Anatomy of which Prof. C. M. Jackson is director, were especially valuable.

It is a great pleasure to the writer to have this opportunity of expressing his obligations to Prof. J. B. Johnston for his valuable suggestions and friendly criticism of this work.

A model of the caudal end of the spinal cord from a region a little in front of the caudal hearts to its extreme tip was prepared in four sections from the 20 cm. Polistotrema series. Also a model in two pieces was prepared of the cavity of the so-called first roof plate expansion and the enlarged central canal of the above mentioned series of Polistotrema. These models were constructed out of blotting paper after a modification of the Born method. Tracings of each section were made on ordinary writing paper with the aid of an Edinger-Leitz drawing apparatus, using a magnification of 100 diameters, and afterward each tracing was carefully checked up for accuracy with a higher magnification. At the very outset two base or projection lines were drawn on the first tracing, one following the median longitudinal plane, and the other a horizontal line drawn at right angles to the first line, passing along the ventral border of the notochord. These projection lines were added by pencil to all of the succeeding tracings after the following manner: The second tracing was carefully fitted over the first, after placing both over a plate glass covered box, containing an electric light reflected upward, and in like manner these lines were added to the third tracing from the second, and so on to the end of the series. Then with the aid of carbon paper these tracings and projection lines were transferred to sheets of blotting paper, having a definite thickness, previously determined after the following method. When thoroughly cooled after an immersion in melted paraffin, the blotting paper selected should have a thickness, equal to the thickness of the section multiplied by the magnification used, which in this case totalled 1.5 mm. The sheets of blotting paper containing the transposed tracings were then immersed in melted paraffin, drained and cooled. After which the outlines of the tracings were cut out with a sharp scalpel, and the sections were built up in regular order. In part to maintain this regular order and in part to add strength to the model, a copper wire was inserted through each section, passing through the point of intersection of the two projection lines. After a certain number of sections had been strung on this wire they were securely fastened to each other by pins.

HEREDITARY AND MECHANICAL CAUSES UNDERLYING THE FORMATION OF THE FOURTH VENTRICLE AND THE TELA CHORIOIDEA

To the writer this problem appears to be primarily phylogenetic rather than ontogenetic; consequently this study begins with the lowest vertebrates, and is approached as a problem of evolution.

1. Roof plate of the medulla oblongata

A. Amphioxus. Unfortunately I have not had access to any embryological Amphioxus material so that my inferences will have to be drawn entirely from adult material.[2] At the outset it can be maintained with a considerable degree of safety that the adult Amphioxus brain contains nothing which can be homologized with the fourth ventricle of higher vertebrates. Figure 67, which pasess through the highest point of the anterior ventricle ($V._1$) demonstrates clearly that there is nothing here comparable with the fourth ventricle. For the dorsal portion of the cavity is much narrower than the ventral portion, and there is absolutely nothing in the way of a thin and expanded roof plate. In fact, the dorsal portion of the cavity is fairly filled up with processes from ependymal cells.

A few sections behind the first ventricle there appears a small isolated cavity in the roof plate region of this series, designated as the posterior ventricle (fig. 68, $V._2$), and a considerable distance behind this cavity, there is a second dorsal cavity, also designated as the posterior ventricle (fig. 69, $V._2$). This is somewhat larger than the previous cavity, and can be readily located from its position immediately above a dorsal group of large nerve cells (*M′.C′.*). From the figures by Hatschek, Willey, Sterzi, and others it is evident that these isolated roof cavities described above, were once a part of a common central cavity of the embryonic brain, which later in development, became isolated through an invasion of ependyma, and it is entirely possible that this region of the central cavity in the embryo was much more suggestive of the fourth ventricle. Judging from the adult alone they may be looked upon merely as vestiges of the embryonic central canal.

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[2] In order to eliminate minor details of description from the text, very complete and detailed descriptions of the figures have been given at the end of the paper, to which the reader's attention is directed.

B. Polistotrema (*Bdellostoma*) My embryonic material of Polistotrema confirms the statements of Price, von Kupffer, and Dean that Polistotrema possesses well-developed ventricles in the embryo; the expansion being fully as great as in a similar stage of Petromyzon. As development proceeds the lateral plates increase in thickness from additions of fibers and cells until the fourth ventricle becomes reduced to a canal, but little larger than the central canal of the spinal cord.

Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls describe and figure the fourth ventricle about as it is shown in figure 63. There is some little discrepancy in the terminology used, due largely to the differences of opinion as to whether or not Polistotrema has a cerebellum. If the posterior lobes of the mesencephalon should in the light of future investigation turn out to be a cerebellum, then the boundaries of the metencephalon will have to be carried further forward than we have indicated, and the so-called sinus mesocœlicus of Nicholls (*S.M.*) will have to be called the anterior dilation of the fourth ventricle of Miss Worthington's description.

My transverse series through the brain of Polistotrema show the condition of the ventricles to be almost identically the same as Sterzi and Nicholls found them. Until the embryological and functional areas of the brain have been better worked out it seems advisable to the writer to let the posterior border of Nicholls' sinus mesocœlicus (fig. 63, *S.M.*) mark the boundry line between the mesencephalon and the metencephalon, and to regard his isthmic and ventricular canals (figs. 63 and 65, *A.₄V.*)

as dorsal and ventral portions of the anterior end of the fourth ventricle. For the reason that in Polistotrema they extend some distance behind the posterior tip of the mesencephalon, and the dorsal or posterior canal is shown in transverse section (fig. 65) to lie close to the dorsal surface, which is then the only part of the fourth ventricle to retain the characteristic dorsal position of the higher vertebrates. These two canals appear in this series about as Nicholls has described them, the dorsal (isthmic) is the larger and contains Reissner's fiber. Although probably subject to a considerable variation, these canals apparently extend further caudad in this series than Nicholls represents them. Also in this series the dorsal canal (isthmic) gives off one or two branches at the level of the posterior tip of the mesencephalon (cerebellum of Miss Worthington), which run parallel to the main canal, but a little to one side and below (fig. 65, $A._4V.$). After travelling side by side for some little distance in a mass of spongy ependymal tissue close to the roof plate, they reunite in the dorsal canal, and soon afterward both the dorsal and ventral (isthmic and ventricular) canals unite in a common canal, which is little if any larger than the central canal of the spinal cord. This constricted portion of the fourth ventricle (fig. 63) continues caudad, rather deep-seated in a mass of loose vascular ependyma, until the posterior end of the medulla is reached, where it expands into a much larger vesicle or sinus, designated as the posterior dilation of the fourth ventricle (figs. 63 and 66, $P._4V$). Behind this it soon tapers down into the ordinary central canal of the spinal cord.

A glance at figures 63 to 66 suffices to show that the fourth ventricle of Polistotrema is greatly reduced as compared with that of Petromyzon. This is due probably to the rapid increase of fibers and cells in the lateral plates. Notwithstanding this reduction in size and general alteration in appearance and structure, the walls of the fourth ventricle in Polistotrema, although representing a greatly modified chorioid plexus, are unquestionably capable of producing cerebro-spinal fluid. The posterior dilation of the fourth ventricle (fig. 66, $P._4V.$) contains cerebro-spinal fluid (*S.C.F.*) in the form of a deeply staining feltwork, which is not ependymal cilia or a tangled Reissner's fiber. Also throughout its entire length, as was noted by Sterzi, the fourth ventricle is enveloped by a rather thick layer of spongy and very vascular ependyma, which would be distinctly favorable for infiltration and possibly for secretion into the ventricle. This rich blood supply is from the blood vessels and sinuses traversing the meningeal membranes, and especially from two large arteries (A. rhombencephalica of Sterzi), one of which appears in figure 64 (*M.A.*). Also it would be quite possible in the anterior part of the fourth (*A.₄V.*) for the various canals, which run close to the dorsal, to receive infiltration direct from the outer meningeal blood and lymph sinuses.

Coagulated cerebro-spinal fluid is also to be seen in reduced amounts in the mesencephalic ventricles designated as the posterior mesocœle and the sinus mesocœlicus. They are also surrounded by a layer of vascular ependyma, which, while much thinner than the corresponding layer of the fourth ventricle, doubtless functions as a cerebro-spinal fluid forming organ.

A careful examination of this peculiar modification of the chorioid plexus of the fourth ventricle in Polistotrema leads one to believe that this is not as efficient an organ for the production of cerebro-spinal fluid as the more expanded tela chorioidea of Petromyzon and higher vertebrates.

C. Petromyzon. Petromyzon is apparently the best type that could be selected for obtaining definite information concerning the early history of the formation of the fourth ventricle and its expanded roof plate. 1) It is the lowest living vertebrate that possesses a well-developed fourth ventricle and expanded tela chorioidea in the adult. 2) At no times does the medulla have a pontine flexure. 3) Its central nervous system remains a solid cord of ectoderm until after the cranial and spinal ganglia are well-differentiated. For these and other reasons the early history of the fourth ventricle in Petromyzon has been studied in the effort to determine the essential factors involved in its appearance and growth.

In order to arrive at the fundamental factors involved in the anlage and development of the fourth ventricle in Petromyzon it is necessary to go back in the ontogeny of the central nervous system to the time when it was a solid cord. Of my embryos which represent stages killed at 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, and 26 days after fertilization, all of the 5 day series revealed the central nervous system as a solid cord or keel of ectoderm cells, formed according to Calberla by a process of delamination. In places neural crest cells can apparently be seen budding off from the dorso-lateral surface of the brain and spinal cord almost identically as described and figured by von Kupffer ('90). It is obvious that the preponderance of yolk granules in all tissues makes accurate observation in these stages very difficult. The majority of the 7 day embryos disclosed a central canal either formed or in the process of formation. Out of a great number of series of 10 day embryos, three were found in which the opening of the central canal had been considerably retarded. In one series the central nervous system was still a solid cord of cells, and in the other two the central canal was in the early stages of formation. These series were selected in preference to similar stages of 7 day embryos for the reason that less yolk was present to obscure the various structures.

To facilitate comparison of similar sections of successive stages, figures 33 to 38 and figures 40 to 53 have been so arranged as to bring several regions of series in a horizontal row, while successive stages of the same region are placed in a vertical row. The conditions at the level of the trigeminal, auditory and vagal ganglia are thus readily compared.

In figure 32 we have a section through the medulla in the region of the VIII ganglion, which portrays very well an extremely early stage in the formation of the central canal. Here the medulla will be seen to be composed of a mass of nuclei imbedded in a syncytium of protoplasm. Excepting in the roof and floor plates, the nuclei have migrated some little distance to either side of the median sagittal plane, presenting the appearance of a rather broad light line surrounded by nuclei. The center of this light streak of protoplasm discloses a very conspicuous furrow or seam (*C.C.S.*), which appears in every section of the central nervous system of this series, very much as it looks in this section. This furrow or seam represents the position of the future central canal. The protoplasm adjacent to this seam is sufficiently granular to suggest a secretory function at this early stage.

Figures 33 to 35 also taken from a 10 day Petromyzon series exhibit a slightly later stage in the formation of the central canal. Conspicuous cavities (*C.C.*) have appeared in the dorsal and ventral portions of this furrow, which are not only visible throughout the entire medulla, but are continuous throughout the spinal cord. It should be recorded that a few sections through the medulla possessed slightly larger dorsal and ventral cavities than were represented by these figures, but in no case had they approached each other close enough to unite. In other respects, excepting possibly for a few more fibers in the marginal layer, the structure of the medulla has remained about the same as in the previous series. Emphasis should be made of the fact that each of these cavities, when examined with a higher magnification, reveals a certain amount of fine granules, which in some cases may have completely filled the cavity, while in others they are confined to the outer edge, leaving a clear space in the center. The presence of these granules here suggests two processes: 1) disintegration of the central protoplasm, and 2) products of secretion. The lateral migration of the nuclei could be utilized to support either inference. That such a migration of nuclei would be favorable for disintegration is self evident, and secretory cells are usually characterized by having their nuclei somewhat remote from their lumina.

In the 11 day series (figs. 36 to 38) we find that the two isolated dorsal and ventral cavities of an earlier stage have not only united and formed a cleft-like cavity, which may now be designated as the typical embryonic central canal, but that the original dorsal and ventral cavities of this canal, especially the dorsal, have increased notably in size. The narrow central portion (figs. 37 and 38) indicates that this is the place where the protoplasm was last to separate. It should not be confused with a similar condition that occurs later, when the central and ventral portions of the lateral plates migrate inward, fuse, and completely obliterate that portion of the embryonic central canal. Aside from the change in the central canal the size of the medulla has increased in all directions, but especially laterally, due doubtless to an increase in the number of nerve fibers and cells in the lateral plates. Both roof and floor plates are very thin, comprised of about one layer of nuclei each. The ventral plate may be slightly thicker, due to the addition of a few nerve fibers to the outer layer. Absolutely no stretching of the roof plate has occurred, indicating that it is not under any marked internal pressure. In figure 37 (*B.V.*) branches of the intersegmental blood vessels have stretched out toward the roof of the brain, but at this stage they are too remote from the roof plate to be very active in infiltration. Figure 39 makes clear that the spinal cord has made equal progress with the medulla in developing a cleft-like or typical embryonic central canal. In the cord the dorsal and ventral enlargements of the central canal are shown to be of about equal size.

An additional day (figs. 40 and 41) discloses considerable expansion of the central canal throughout, with the exception of the extreme ventral portion. Very noticeable is the increase in size of the dorsal portion, the future fourth ventricle (cavità della tela coroidea of Sterzi). The roof plate exhibits no signs of stretching. Up to this stage the expansion appears to be due to the migration of the ependymal cells upward and outward, rather than to pressure within. The floor plate will be seen to have increased considerably in thickness through an addition of fibers to its marginal layer, which would obviously tend to make the floor plate more resistant than the roof plate to pressure from within from this time on. Both lateral plates disclose a remarkable growth in thickness due to an addition of both fibers and cells, some of which, however, must be attributed to the fact that the head was sectioned somewhat obliquely (note in fig. 40 that the V and VIII ganglia appear in the same section). But little progress has occurred in the development of the intersegmental blood vessels, so that Sterzi's conjecture, that the embryonic cerebro-spinal fluid does not differ in any way from the ordinary intercellular fluids, would probably hold, if it could be established that the central protoplasm in disintegration contributed nothing to it. The spinal cord was found to be in about the same condition as the 11 day series.

Moreover an 18 day series (figs. 42 to 44), some 6 days older than the last stage compared, shows practically no change in the shape and size of the central canal or expansion of the roof plate, despite the fact that a notable increase of fibers had occurred in the ventral and median portions of the lateral plates. It should also be recorded that the intermediate stages, as represented by the 14 and 16 day series, exhibited a like state of the central canal. A further interesting condition is revealed from a section of the spinal cord of this series (fig. 45). Here the lateral plates have expanded to such an extent from the formation of fibers in the marginal layer, that the inner surfaces of their central areas have nearly formed a complete concrescence at the center of the original central canal. In other words, the inner surfaces of the lateral plates have met, and are about to fuse, leaving dorsal and ventral cavities (*C.C.*). The ventral one of these cavities will persist as a permanent central canal. In Petromyzon this partial closure of the original embryonic central canal of the spinal cord may have considerable bearing on the increase of internal pressure of cerebro-spinal fluid on the walls of the medulla, during this and later stages. We may regard the period from about the 12th to about the 18th day as a period of rest in the formation of the fourth ventricle in Petromyzon. During this time the processes which would tend to expand the central canal have been met with equal counter forces, which would make for closing it up.

Transverse sections through the medulla of a 20 day Petromyzon series (figs. 46 to 48) demonstrate a marked change in the medulla and its central cavity, which cavity has now assumed the form of a fourth ventricle. A marked increase has taken place in the number of fibers in the ventral plate and in the ventral portion of the lateral plates. This together with pressure from the growing auditory vesicles (*Aud.V.*) and the notochord, has produced a nearly complete concrescence of the corresponding middle and ventral portions of the inner surfaces of the lateral plates. Had a similar increase of nerve fibers occurred in the dorsal plate and in the dorsal portion of the lateral plates, the central canal of the medulla might have been completely obliterated. On the contrary, the dorsal cavity and the roof plate have been slightly expanded, apparently through an increased internal pressure from the embryonic cerebro-spinal fluid. One evidence that increased pressure may exist was found in the marked decrease in the size of the central canal of the cord and of the ventral part of the canal in the medulla region.

A second evidence is found in the changes that the walls of the fourth ventricle are undergoing preparatory to becoming a functional organ for the production of cerebro-spinal fluid. In connection with the expansion of the roof plate it will be seen that the dorsal mesenchyme has become decidedly vascular (fig. 46, *B.V.*), making easy an infiltration process into the ventricle. Also ependymal cells, surrounding the ventricle, are taking on form and are probably assuming a secretory function, if they have not acquired one previously. From the recent work of Dandy and Blickfan based on the action of certain drugs and on the chemistry of cerebro-spinal fluid, it is evidence that cerebro-spinal fluid must be a product of secretion as well as of infiltration and diffusion. It is a well-established physiological fact that certain secretory cells, as for example the salivary glands, may assume a definite polarity, and produce a secretion against a very strong pressure, even stronger than that of the blood. Some investigators hold that lumina in glands are the result of pressure from secretion.

In the light of these facts, it is fair to assume that the marked lateral expansion of the fourth ventricle and its roof plate exhibited in the 26 day series (figs. 50 to 52) are the direct result of internal pressure caused by the marked increase in cerebro-spinal fluid. The general increase in size of the ventricle together with the marked convexity of the roof plate and the concavity of the internal surface of the lateral plates certainly suggest internal pressure from the cerebro-spinal fluid. It should be noted in figures 50 to 52 that this expansion of the fourth ventricle has produced a considerable secondary splitting in the concrescence of the lateral plates recorded for the previous series. Apparently this fissure did not penetrate so deeply in the region of the auditory vesicles (fig. 51, *Aud.V.*) as it did in front of them (fig. 50) or behind them (fig. 52), which probably indicates that the growth of the auditory vesicles in some way operated against the further splitting of medulla. It is significant that the expansion of the roof plate and the development of its mechanisms for infiltration and secretion occur at a time prior to the entrance of blood vessels into the wall of the brain and cord. The large production of cerebro-spinal fluid at this time is evidence that it serves some nutritive function.

In figure 54 we have a median sagittal section through the brain of a 26 day Petromyzon, representing a stage similar to that of figures 50 to 53. Especial attention is called to the fact *that figure 54 demonstrates conclusively that the pronounced roof expansion displayed in figures 50 to 52 has occurred without the aid of a pontine flexure.* The slight convexity of the floor of the medulla can be attributed to the increase of fibers. Also earlier and later series revealed that Petromyzon possesses no pontine flexure. Attention should be called to the fact that the marked convexity of the cephalic end of the thin roof plate of the fourth ventricle (*C.C.*) in figure 54 gives every appearance of being under internal pressure from cerebro-spinal fluid. This section also makes clear, as Sterzi has previously shown, that the fourth ventricle is formed from the dorsal portion of the embryonic central canal; while the central canal of the cord is formed from the ventral portion.

The following observations were noted in connection with the appearance of the fourth ventricle in Petromyzon, beginning at a stage when the medulla was a solid cord of undifferentiated nuclei in a syncytium of protoplasm: 1) The ependymal nuclei migrated a short distance to either side of the median sagittal plane leaving a narrow strip of granular protoplasm in the center. 2) A median sagittal furrow or seam appeared in the central protoplasm, extending from the roof plate to the floor plate. 3) An isolated cavity appeared at the dorsal and ventral ends of this furrow. 4) The two cavities became connected, forming a cleft-like canal, designated as the typical embryonic central canal. This canal seems to be formed by a disintegration of the central ends of the ependymal cells, now in the form of a syncytium. 5) A considerable increase in the size of the dorsal portion of the central canal occurred through the upward and outward migration of some of the roof plate nuclei and a disintegration of the inner protoplasm. No similar expansion of the ventral portion of the central canal of the spinal cord took place because its marginal layer became reinforced very early by the addition of nerve fibers. 6) Following the formation of the central canal of the medulla there was an increase of cells and fibers in the lateral walls, but for the space of about six days there was little change in the size of the central canal. 7) Next a very pronounced increase of fibers occurred in the median and ventral portions of the lateral plates, which brought about a complete concrescence of the corresponding inner margins of the lateral plates. 8) A sufficient amount of cerebro-spinal fluid was formed by infiltration and secretion to produce a marked expansion of the fourth ventricle. This expansion pushed apart the dorsal portion of the lateral plates, which had not been thickened by an addition of fibers, and stretched the roof plate to a much greater width. 9) Along with this expansion a secondary splitting of the concrescence noted in (7) took place. This fissure did not penetrate so deeply in the region of the auditory vesicles on account of the mechanical obstacle offered by these vesicles. 10) This expanded roof plate apparently assumed the function of producing cerebro-spinal fluid at a time previous to the entrance of blood vessels into the central nervous system, when its nutritive function would be of importance.

From a review of the main points in the development of the fourth ventricle in Petromyzon we are warranted in concluding: 1) That a well-developed fourth ventricle and tela chorioidea were formed in one of the lowest living vertebrates, Petromyzon, without the aid of a pontine flexure. 2) The best suggestion that has been given for the appearance of such an organ in the medulla rather than elsewhere in the central nervous system is that the roof plate has been weakened more in the medulla region through a greater migration of neural crest cells. This explanation, however, is not entirely satisfactory; since it was shown for Petromyzon (fig. 32) that the cranial ganglia were well-differentiated while the medulla was still a solid cord of cells. If then this were the determining factor it must have cast its shadow a long way ahead. Also it is apparent when the difference in size is considered between the medulla and the spinal cord that there are relatively no more neural crest cells extruded from the medulla. 3) The roof expansion appeared at the same time in the region of the X ganglion as in the region of the VIII and V, and developed at a uniform rate throughout the medulla. 4) Two factors are evident in the formation of the fourth ventricle; first, an upward and outward migration of the roof plate nuclei followed by a disintegration of the inner protoplasm; second, internal pressure exerted by the rapidly increasing cerebro-spinal fluid, infiltrated and secreted by the roof plate itself.

D. Selachians and Amphibia. As types of these classes I had access to very complete sets of serial sections of Squalus and Amblystoma embryos, and to one transverse series of a 15 mm. Necturus. A careful examination of this material contributed nothing new to the ontogeny of the fourth ventricle. It was possible, however, to confirm in these forms many of the conclusions arrived at in Petromyzon. In both Squalus and Amblystoma the much earlier appearance of the central canal brings about a much earlier and more extensive expansion of the fourth ventricle. The sections shown in figures 72 and 73, while possessing enormous expansions of the roof plate, exhibit less progress in the differentiation of the structure of the medulla than was revealed in a 12 day Petromyzon (fig. 41), in which very little or no expansion had taken place in the roof plate.

In Squalus and Amblystoma the typical embryonic central canal, in the form of a vertical cleft, is a comparatively late production. The canal appears soon after the neural folds coalesce as a horizontal cleft, its presence often being indicated only by a layer of pigment. This cleft becomes elliptical, then more or less circular, and finally changes to a vertical or dorso-ventral cleft. These changes in the shape of the central canal suggest a migration of cells, rather than a disintegration process or a splitting apart of the walls as a result of pressure from the secretion of cerebro-spinal fluid. The earliest Squalus series showed an elliptical canal with its longest axis horizontal, this changed to circular, and finally to the so-called typical embryonic central canal (dorso-ventral cleft canal). For a time in the medulla of Amblystoma the roof plate is much thicker than the floor plate, a condition which can be attributed to the presence of a number of neural crest cells in the roof plate, easily distinguishable from the other cells by their large size and spherical form. After the neural crest cells had been entirely separated from the roof, the roof and floor plates are found to be about equally thick. The same thinning out of the roof plate occurs in Squalus in part through the giving off of neural crest cells, which in this species are indistinguishable from the other cells of the medulla. It is evident in Amblystoma (figs. 74 and 83, *R.P.*) and also in Squalus that the roof plate of the medulla has become no thinner than the roof plate of the spinal cord through the throwing off of the neural crest cells. There is no evidence that the roof plate in the medulla is rendered any weaker or any more susceptible to expansion than is that of the spinal cord, through the migration of ganglion cells.

Apparently the first expansion of the roof plate in Amblystoma (fig. 74, *R.P.*) and in Squalus is produced as in Petromyzon by an outward and upward migration of the roof plate cells. The later, more pronounced expansion and stretching of the roof plate can also be ascribed, as in Petromyzon, to internal pressure due to a decided increase of cerebro-spinal fluid (see fig. 72 for Squalus and 73 for Necturus). In both species the dorsal and middle portions of the embryonic central canal of the spinal cord are obliterated by an inward growth of the lateral plates through an addition of fibers. Pressure on the cerebro-spinal fluid might be increased from that source. Also a similar effect would be produced through a marked proliferation of fibers in the medial and ventral portions of the lateral plates of the medulla, which brings about a coalescence of the ventral portions of the lateral plates in Squalus (fig. 72, *C.C.C.*) similar to the condition in Petromyzon. In Necturus (fig. 73) and in Amblystoma the roof plates are expanded to such an extent that no fusion of the lateral plates takes place; nevertheless, the thickening of the walls tends to bring about a reduction in the size of the fourth ventricle and consequent pressure on the cerebro-spinal fluid. The probability of a decided increase of internal pressure from the cerebro-spinal fluid resulting from the reduction in caliber of the embryonic central canal of the spinal cord by at least two-thirds, is more evident, if attention is directed to the fact that the length of the central canal of the spinal cord is fully twenty times that of the fourth ventricle. In both forms the above changes took place before the blood vessels had reached the dorsal surface of the roof plate or entered the medulla. A median sagittal section through a Squalus embryo of the same stage as figure 72 shows that no pontine flexure has appeared.

E. Pig embryos. For making observations on the development of the tela chorioidea of the fourth ventricle in mammals the writer had access to a very complete set of frontal sections of pig embryos from a stage of 4 or 5 mm. up to 14 mm. embryos.

The earliest section (fig. 75), which is from a 4 or 5 mm. pig, discloses that the fourth ventricle has only begun to expand. A large part of this expansion could be attributed to an outward and upward migration of the roof plate cells, and a part to internal pressure from cerebro-spinal fluid, of which traces are beginning to appear as a coagulum (*S.C.F.*). It should be noted that the first blood vessels are appearing above the basal portions of the roof plate, while none have at this stage entered the medulla. Also the ependymal cytoplasm is sufficiently granular to suggest a secretory process, and finally the roof plate has begun to expand before any nerve fibers have appeared in the marginal layer.

A transverse section of the medulla of a 6 mm. pig (fig. 76) revealed a considerable expansion of the roof plate without the aid of a pontine flexure. It is apparent that an increase in the amount of cerebro-spinal fluid is the main factor in bringing about this pronounced expansion of the roof plate. Since the closure of the dorsal portion of the embryonic central canal of the spinal cord occurs much later in embryonic life, no increase in pressure from cerebro-spinal fluid could take place from that source. Also no increase was shown in the number of blood vessels outside the roof plate, and no blood vessels had entered the medulla. Consequently the only means of an increase of cerebro-spinal fluid would be through secretion and a slight infiltration from the blood vessels. This section demonstrates very strikingly, even more so than figure 75, that the roof expansion begins very early in the development of the pig's medulla, as is evident from the fact that nerve fibers have only begun to appear in the marginal layer.

Between the 6 mm. and 7 mm. stages there occurs a marked increase in the blood vessels in the mesenchyme above the roof plate and a few blood vessels are entering the substance of the medulla. There can be no question that the roof plate is now an efficient organ for the production of cerebro-spinal fluid, and there is a noteworthy increase in the amount of coagulum in the cavity (figs. 75, 76, and 77). The first embryonic cerebro-spinal fluid, probably a mere intercellular fluid, showed little or no coagulum, from the method of fixation and staining used. This may indicate a relative increase of the elements formed by secretion. Figure 77 shows that a marked expansion of the roof plate has occurred in the 7 mm. pig. Since the 7.5 and 8 mm. pig embryos have very small pontine flexures, it is evident that this pig embryo has developed a well-expanded roof plate and chorioid plexus without the influence of a pontine flexure.

The roof plate in the 10 mm. pig (fig. 78, *R.Ex.*) and in the 14 mm. pig (figs. 79 and 80, *R.Ex.*) has undergone a decided expansion, especially in a dorsal direction. This final expansion of the roof plate is unquestionably due to the action of the pontine flexure upon a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure. It would have been impossible for a pontine flexure acting alone on an empty fourth ventricle, as would be implied from His' experiments with bending an empty rubber tube slit dorsally, to have brought about the dorsal expansion of the roof plate exhibited in figures 79 and 80. Apparently this expansion of the roof plate in the pig has been gradual, for absolutely no stretching of the roof plate has occurred, except in one place, namely in its central anterior portion (fig. 79).

A study of these sections has disclosed a direct relationship between the expansion of the roof plate and the amount of visible coagulum in the ventricle. Since coagulum does not appear in sections of the early fourth ventricle, but does appear after the tela chorioidea has attained the function of producing cerebro-spinal fluid (as is indicated by its vascularity and the granular appearance of the cells) it is fair to assume that the non-coagulable cerebro-spinal fluid found in sections of the early embryos is an embryonic cerebro-spinal fluid, which differs in no way from the ordinary intercellular fluid of other tissues. On the other hand, the coagulum seen in sections after the roof plate has reached the stage of a functional chorioid plexus is evidence of a chemical change in the fluid, which, if a product of secretion, is capable of exerting considerable internal pressure and consequent expansion of the roof plate.

It is apparent that the greater expansion of the roof plate in the pig is produced by the same factors as were recorded for Petromyzon, namely, an early migration outward of the roof plate cells followed by an expansion from within due to the formation of cerebro-spinal fluid, plus the action of a conspicuous pontine flexure on a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure.

F. Human embryos. For this study an 8 and a 15 mm. transverse series and a 23 mm. frontal series were available. These embryos were too far advanced to show the earliest stages of the roof expansion of the fourth ventricle. If, however, the extreme posterior end of the roof plate of the fourth ventricle is examined in the 23 mm. embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. embryo (fig. 31, *R.Ex.*), which represents a region of the medulla little affected by the pontine flexure, it is apparent that the roof expansion was caused by identically the same factors as was recorded for the pig embryos. In view of the facts, this explanation of the formation of the roof expansion seems more tenable to the writer than to attribute all, as His has done, to the action of a pontine flexure. For beyond question, a considerable expansion of the roof plate in the human medulla takes place before the pontine flexure appears.

2. Description of three roof plate expansions of the spinal cord in the 20 cm. Polistotrema series

An interesting variation (abnormality) was found in the spinal cord of a single specimen of Polistotrema (Bdellostoma), otherwise unusual. Certain structures appeared in the roof of the cord that were very similar to the tela chorioidea of the fourth ventricle, and will be described because of the light that they may throw on the origin of the tela chorioidea of the fourth ventricle.

What has been designated as the first roof plate expansion of the 20 cm. Polistotrema series is shown in the photographs of models 1 and 2 (figs. 1 and 2, 4 and 5, *R.Ex.*) to be an immense outcropping of the roof plate ependyma. Most unfortunately the anterior portion of this specimen, from which the series through the tail was taken, has been lost, so that it is impossible to state how much further cephalad this expansion of the roof plate extended, or whether there were other outcroppings of the roof plate in front of it as there are behind. It appears in the first model (fig. 2, *R.Ex.*) and in the first transverse section (fig. 10, *R.P.Ex.*) as a median mass, covering about one-half of the dorsal surface of the spinal cord; it then shifts gradually over to the right side (figs. 2 and 11); then gradually attains a median position. In this position it continues as far as the middle of model 2 (fig. 5), covering a large part of the dorsal surface of the spinal cord.

For the most part the roof plate expansion contains a cavity of considerable size, which is shown anteriorly in the cast (fig. 3, *C.C.Ex.*) and in transverse section (figs. 10 to 12) to be in direct communication below with the central canal. Posteriorly such a connection is wanting (fig. 13). The fact that in various places (fig. 13) there is more or less of a string of ependymal cells between the walls of the central canal and the roof plate expansion suggests that in a more embryonic state an open communication existed between the roof plate cavity and the central canal, which became closed in the region of the posterior end of the roof expansion, and persisted in the anterior end. Another variation to be noted in the cavity of the posterior end of the roof plate expansion is that it contains numerous islands and promontories of ependymal cells and connective tissue, shown as white spaces in figure 6. These probably represent portions of the roof plate that have not been completely excavated to form a continuous cavity.

Transverse sections 11 to 13 show the cavity of this first roof plate expansion to be larger than the fourth ventricle in Polistotrema (figs. 64 to 66), and the whole structure more nearly resembles a typical fourth ventricle than does the fourth ventricle itself in this animal. For the most part the walls of the roof plate consist of true ependymal cells, differing in no way from those surrounding the central canal, except for their shorter peripheral processes. Posteriorly connective tissue takes the place of many of these cells. One of these cells is shown in figure 13A to be sufficiently granular to suggest a secretory function. Everywhere the walls of both the roof plate and the central canal are very vascular, suggesting a modified choroid plexus. Figure 13A will demonstrate the ease with which infiltration and diffusion could take place between the blood vessels of the roof plate and its cavity. In figure 11 a fold of the roof expansion, containing a blood vessel, will be seen extending into the cavity, and about it there is collected a mass of coagulated cerebro-spinal fluid (*S.C.F.*).

Of still greater interest are the two posterior outcroppings of the roof plates, designated as roof plate expansions 2 and 3 (figs. 4 and 5, *R.Ex. 2 and 3*), Since these two roof plate expansions are considerably smaller than the first, they can be compared directly with the roof plate of the rhombencephalon of any embryo.

The so-called second roof plate expansion appears in model 2 (figs. 4 and 5, *R.Ex. 2*) immediately behind the first. This is a much smaller outcropping of the roof plate. It contains a cavity (figs. 16 and 17, *C.C.Ex.*), which spreads out a little laterally and caudally, and communicates below with the central canal. This cavity is filled with a fine fibrillar feltwork that stains deeply with orange G, and which for the most part is coagulated cerebro-spinal fluid. The ependeymal walls of both the roof expansion and the central canal are sufficiently vascular to suggest that we have here as in the previous roof expansion, a modified chorioid plexus which is producing cerebro-spinal fluid.

In model 2 (figs. 4 and 5, *R.Ex. 3*) the third outcropping of the roof plate is some little distance behind the second, about equaling it in size. This roof expansion has not been figured in transverse section, but from an examination of a graphic reconstruction of the central canal, three small isolated cavities were seen extending in a cephalo-caudal direction. The middle cavity was found to be in communication below with the central canal. It held cerebro-spinal fluid, and its walls apparently functioned in the production of the same.

It is evident that these three expansions of the roof plate are independent of one another. The arrangement of the three small isolated cavities in the third expansion seems to be an embryonic condition, and suggests that the larger cavities may have been produced by the union of several smaller roof expansions. It may be supposed that these expansions were formed by the multiplication of roof plate cells, which were pushed up in solid masses, in which vacuolization and confluence of adjacent cavities produced the larger cavities seen in the adult.

It is of interest to note that in this individual a posterior sinus (fig. 20, *S.T.*), probably representing the sinus terminalis of a normal individual, was isolated by the complete occlusion of the central canal by ependymal tissue. The ependyma surrounding this cavity is very vascular, and the cavity is distended with cerebro-spinal fluid. This sinus is much larger than the fourth ventricle in a normal individual.

When the roof expansions in the spinal cord of this series of Polistotrema are compared with the fourth ventricle of the higher vertebrate embryos, it is evident that the similarity is only superficial, for the later stages in the medulla oblongata (action of pontine flexure on a thin-roofed neural tube full of cerebro-spinal fluid under moderate pressure), are dependent on factors which are not present in the spinal cord of Polistotrema. The extreme caudal end of the roof plate of the fourth ventricle in the higher vertebrates, however, has not been affected by these later factors, and presents a condition where comparison is made possible. From a comparison of the roof plate expansion in the 23 mm. human embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. human embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. human embryo (fig. 31, *R.Ex.*) with the second roof plate expansion of the spinal cord in the 20 cm. Polistotrema series (figs. 4 and 5, *R. Ex. 2*, and figs. 15 to 17, *R.P.Ex. 2*) and with the third roof plate expansion of the same series (figs. 4 and 5, *R.Ex. 3*), it is evident that the same main factor is present in all, namely, a migration outward and upward of certain roof plate ependymal cells to form an enlarged dorsal cavity. In each case the purpose of this structure is to form an organ for the production and storing of cerebro-spinal fluid. As soon as these structures assumed the function of infiltrating and secretory organs their walls became further expanded from internal pressure of the cerebro-spinal fluid.

From a review of the early stages of the formation of the roof expansion in man, the pig, Amblystoma, Squalus, and Petromyzon, it is fair to assume that the source and early development of the roof expansion of the medulla are identical to the three similar roof plate expansions, described for the spinal cord of a 20 cm. Polistotrema. It is possible that both had their *phylogenetic anlage* as mutations, the former from some primitive vertebrate, and the latter from a normal Polistotrema, that both were useful and dominant characters, and in case of the medulla, where the animal was allowed to reproduce, this character became preserved for the race.

CAUSES UNDERLYING THE FLATTENING OF THE SPINAL CORD IN CYCLOSTOMES

If our attention is first directed to a transverse section through an adult Polistotrema (figs. 10 and 71) it might be inferred, since there is ample room in the membranous neural canal for a well-rounded spinal cord, that the flattening of the spinal cord might be attributed entirely to internal factors. A glance at a transverse section through a developing Polistotrema spinal cord (fig. 57) suffices to show that there is proportionately much less room within the membranous neural canal, and that a mechanical force in the form of a rapidly growing notochord is at work immediately below the spinal cord.

At the outset it seems advisable to establish arbitrarily a typical state of an embryonic spinal cord, by which a direct comparison of one form can be made with another. The examination of very early stages of the spinal cord in a large number of embryos of Squalus, Amblystoma, the chick, and the pig, in all of which the neural tube is formed by the rolling up of the neural plate, shows that the neural tube passes through three stages: a) A depressed tube with the central canal in the form of a horizontal cleft; b) a cylindrical tube with the canal circular in cross section; and c) a laterally compressed tube with the canal in the form of a vertical cleft (figs. 81 to 86). The existence of this series of changes in Squalus has been shown in a table of developmental stages compiled by Scammon. The third stage may be selected as the typical embryonic spinal cord. This stage is reached at about the time of the first appearance of nerve fibers in the marginal layer.

As a result of a comparison of the typical embryonic stages of the spinal cord in the following transverse sections[3] (fig. 39 for Petromyzon, fig. 55 for Polistotrema, fig. 81 for Squalus, fig. 83 for Amblystoma, fig. 84 for the turtle, fig. 85 for the chick, and fig. 86 for the pig) it is clear that we have all gradations from the nearly cylindrical spinal cords of the Cyclostomes (Petromyzon and Polistotrema) to the very much compressed (flattened laterally) cords of the chick and pig. Were it not for the intermediate stages of Amblystoma and the turtle we might be justified in establishing two distinct types of the embryonic spinal cord: type 1 cylindrical, and type 2 compressed. We could even go further and classify the Cyclostome embryonic cord under type 1 and the Gnathostome cord under type 2.

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[3] It is obvious that this comparison would have no value unless the sections were truly transverse sections. To avoid selecting oblique transverse sections, these figures were always drawn from anterior trunk sections if the embryo showed any flexures.

While the equal expansion of the spinal cord through the addition of nerve cells and fibers to the typical embryonic stage in the pig would tend to produce a cylindrical spinal cord, and in the Cyclostome would tend to produce a depressed cord, the internal structure shows that the origin of the differences between the spinal cord of a Cyclostome and a mammal is not so simple. The neural axis of Petromyzon in an early stage, corresponding to stage (a) above, is decidedly compressed instead of depressed. The problem then is to explain the change from a laterally compressed cord in the early Petromyzon embryos to the gradually depressed, ribbon-like, spinal cord of the adult.

A careful examination of figures 55 to 57, which are taken from practically the same region from three different Polistotrema embryos, shows very clearly that the growing notochord is bringing about the marked flattening (depression) and ventral indenting of the spinal cord. In connection with figure 55 it should be noted that the spinal cord is in the so-called typical embryonic stage, and amid surroundings peculiarly favorable for undergoing a depression from a rapidly growing notochord. It will be seen that the spinal cord is closely enveloped by a meningeal membrane (*P.M.*), approximating a layer of connective tissue, the future membranous neural arch, which is firmly attached below to the growing notochord. Directly above, the mesenchyme is proliferating and migrating toward the center to form a median dorsal cartilage (*M.D.C.*); while there is apparently but little lateral resistance in the way of massing of mesenchyme and the formation of myotomes, In a somewhat later stage (fig. 56) some growth has taken place in the notochord, producing a slight indentation on the spinal cord. In a much later stage (fig. 57) a median dorsal cartilage has been formed. The much stronger membranous neural arches are firmly attached above to this dorsal cartilage and below to the notochord. The soft plastic spinal cord is thus closely confined by the dorsal cartilage above and less closely by the neural arches laterally. The notochord beneath it has grown very rapidly and its enormous increase in size has brought about a decided flattening (depression) of the spinal cord and indentation of its ventral surface.

It is equally clear in Petromyzon also that the growing notochord is to be looked upon as a direct cause for bringing about the flattening of the spinal cord. The external conditions surrounding the spinal cord are shown in figure 58 to be equally favorable for assisting the notochord in this process, with the possible exception that the membranous neural arch is attached above to a membranous neural spine instead of a cartilage, which, however, may be compensated for by an increased dorsal growth of the myotomes.

The hypothesis that the flattening of the spinal cord in Cyclostomes is largely brought about by the upward growth of the notochord after the manner set forth in the previous paragraph is considerably strengthened by the fact that a certain relationship exists between the size of the notochord and the amount of flattening of the spinal cord.

This is clear in the 20 cm. Polistotrema series from sections of the posterior end of the spinal cord (figs. 19, 21, and 22) and from the photographs of the model of the same region (figs. 7 to 9); the flattening (depression) of the spinal cord becoming less evident as the notochord decreases in size. More striking is a similar relationship shown in the tail region of the 70 mm. Polistotrema (figs. 59 and 60) for the reason that these two sections were only one-fourth of a millimeter apart. The above relationship between the size of the notochord and the depression of the spinal cord can be demonstrated fully as conclusively in the medulla region (see figs. 61 and 62). It should be recorded for these two sections that their structure is the same as that of the spinal cord and that they are less than one-half a millimeter apart. Also the same relationship could be shown in sections anterior to figure 62, and from similar sections of a Petromyzon larva. A possible objection to applying this argument in the tail region might arise from the fact that the extreme posterior end of the spinal cord is non-nervous, consisting entirely of ependyma and undifferentiated embryonic cells. In reply to this we would invite comparison of figures 21 and 22, where the structure is non-nervous in both cases, and where the effect of the notochord is obvious.

That the spinal cord of the higher vertebrates has not been depressed by pressure from the notochord is due obviously to the fact that the notochord is an embryonic structure, which never attains sufficient size to have any influence on the spinal cord. This is clear from figures 85 and 86. In Cyclostomes, however, the notochord is a very important structure, develops early and grows for a long period of time, and serves as the skeletal axis of the adult. In fishes, Amphibia, and reptiles the growing notochord may have some slight effect in flattening of the adult spinal cord. In Amphioxus the ventral surface of the spinal cord clearly shows the indenting effect of the growing notochord (fig. 70), and in the trunk region where the diameter of the notochord is greatest, the spinal cord is most depressed.

GENERAL CONSIDERATIONS AND SUMMARY

From the foregoing facts the following conclusions seem fully warranted:

1) In the development of the roof plate expansion (tela chorioidea) in the medulla oblongata of most vertebrates three separate stages or epochs of expansion should be recognized: a) A first enlargement of the dorsal portion of the embryonic central canal took place from a migration outward and upward of certain of the roof plate cells, or as was the case in Petromyzon from the migration of the nuclei and probable disintegration of the cytoplasm. b) The second stage in the expansion of the roof plate was the direct result of an increase of pressure from the cerebro-spinal fluid, produced from at least two possible

sources. First in the lower vertebrates, as a consequence of considerable embryonic cerebro-spinal fluid being forced into the fourth ventricle from the closure of the dorsal and central portions of the embryonic central canal in the spinal cord and the ventral portion of the embryonic central canal in the medulla, through the union and fusion of the corresponding portions of the lateral plates. Second, in all vertebrates, through the production of cerebro-spinal fluid by the walls of the fourth ventricle assuming the rôle of infiltration, diffusion and secretion. That the ventral portion of the embryonic central canal of the spinal cord was not expanded by the same cause is explained by the fact that it was reinforced at a very early stage by nerve fibers and supported by a growing notochord. c) A third and final stage in the expansion of the roof plate in the higher vertebrates was brought about by the appearance of a pontine flexure acting on a thin-roofed medulla filled with cerebro-spinal fluid, itself under moderate pressure.

2) It is the opinion of the writer that the most important factors in bringing about the expansion of the roof plate of the medulla are those concerned in the second stage described above. For a considerable period of time these factors apparently work in conjunction with the forces of the first stage in resisting counter ingrowths of the lateral plates which would tend to close up the central canal. Since it was shown that no pontine flexure occured in Petromyzon, it can be concluded that the rather extensive expansion of the roof plate in the medulla of this genus was accomplished solely through the factors entering into the first and second stages. For the second stage it was recorded in most cases, especially well shown in the pig, that the size of the fourth ventricle and the expansion of its roof plate bear a close relationship to the amount of coagulum seen in sections. Since this coagulum did not appear in sections until after the roof plate had attained the function of producing cerebro-spinal fluid, it probably indicates a chemical change in the cerebro-spinal fluid, the products of secretion now being added to the tissue fluids which entered the ventricle by infiltration. As a product of secretion, the cerebro-spinal fluid would be capable of exerting

considerable pressure on the thin and plastic roof plate. The tela chorioidea is differentiated as an organ for the production of cerebro-spinal fluid before blood vessels have entered the central nervous system, at a time when the nutritive function of this fluid is important.

3) The appearance of a third stage in the roof expansion of the medulla, due to a pontine flexure, is of little significance save in the higher vertebrates, where it was held that without cerebro-spinal fluid confined in the ventricle there would be no reason for maintaining that a further expansion of the roof plate would take place from the action of a pontine flexure; more than likely, the roof plate would have been folded up within the ventricle. In His' experiment with the bending upward of a dorsally-slit piece of rubber tubing, the elasticity of the rubber tubing, which forced apart the cut surfaces, would be comparable to the action of the cerebro-spinal fluid under moderate pressure within the ventricle, which factor His has apparently disregarded.

4) In the adult Amphioxus there is nothing which for a certainty could be homologized to the fourth ventricle and its expanded roof plate. Two isolated cavities in the region of the roof plate, which might be taken for the anlage of the fourth ventricle, appear to the writer to be nothing more than vestiges of a much larger embryonic central canal. If Amphioxus possesses no fourth ventricle in the adult we may safely conjecture that more primitive vertebrates had a central nervous system in which there was no distinction between medulla and spinal cord.

5) In an attempt to trace the phylogenetic history of the roof expansion of the fourth ventricle in living vertebrates, the peculiar modification of the fourth ventricle in the adult Polistotrema (Bdellostoma) should be recorded here even though it has been accurately described by Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls. From the adult it is evident that the well-formed fourth ventricle of the embryo has become transformed through a process of centralization to a deep-seated canal, for the most part no larger than the central canal of the spinal cord. Of especial interest is the fact that its anterior and pos-

terior portions has developed into specialized organs for the production of cerebro-spinal fluid. Notwithstanding this specialization, the fourth ventricle is thought to be decidedly inferior to the tela chorioidea of Petromyzon as an organ for the production of cerebro-spinal fluid.

6) In the spinal cord of one individual of Polistotrema there occurred at least three expansions of the roof plate which resemble the roof of the fourth ventricle in other vertebrates. From the fact that these expansions were very vascular and their cells granular it is inferred that they functioned as choroid plexuses for the formation of cerebro-spinal fluid. The writer presents the hypothesis that the fourth ventricle in ancestral vertebrates may have originated as a mutation, similar to this sport plexus in the spinal cord of Polistotrema; that such sport expansions may have occurred at various places such as the diencephalic segment, the roof of the mesencephalon where a choroid plexus still exists in Petromyzon, and in the hind brain and spinal cord. Such mutations, proving to be useful have been preserved in the vertebrate race.

Concerning the flattening of the spinal cord in Cyclostomes

7) A great variation in the shape of the so-called typical embryonic spinal cord is to be recorded. In Petromyzon it was found to be nearly cylindrical, to be moderately compressed in Squalus, Amblystoma, and in the turtle, and decidedly compressed in the chick and the pig.

8) To obtain this typical stage the original compressed spinal cord of Petromyzon must have undergone a marked depression, and the early depressed neural tubes of Squalus, Amblystoma, turtle, chick, and pig must have undergone a decided compression. The main factor causing this depression in the former was thought to be ventral pressure from a growing notochord, and the compression of the latter was attributed to lateral pressure from the growing myotomes.

9) Transverse sections immediately before and during the time that the greatest depression of the spinal cord is taking place

in Polistotrema and Petromyzon show conclusively that the main factor involved is the pronounced growth of the notochord. It was further established that the embryonic spinal cord was not only in a very plastic condition, but that the general environment was decidedly favorable for bringing about a depression of the spinal cord through this agency.

10) The conclusions outlined in (9) were considerably strengthened by the fact that a direct relationship was established, in both the medulla and tail region, between the size of the notochord and the amount of depression exhibited in the spinal cord. This was shown in late embryos in both Polistotrema and Petromyzon, and in adult Polistotrema.

11) That a similar depression did not take place in the higher vertebrates from a growing notochord was explained by the fact that the notochord is relatively a transitory and insignificant structure; while in the Cyclostomes it is not only formed early in embryonic life, but grows rapidly and continuously.

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PLATE 1

EXPLANATION OF FIGURES

1 to 9 represent photographs from models of the extreme posterior end of the spinal cord from a 20 cm. Polistotrema (Bdellostoma), illustrating a series of three extensions of the central canal into roof plate expansions. These models were prepared at a magnification of 100 diameters, and were reduced one-half in photographing. In a few cases where certain outlines were somewhat indistinct in the photographs they were strengthened with pen and ink. The roof plate expansions in these models were painted lighter and appear the same in the photographs. Except at the posterior end of the last model, all of the models of the spinal cord exhibit a marked depression and in ventral views they show a pronounced indentation at the center immediately above the notochord. As indicated by an arrow, the caudal direction in figures 1 to 3 is toward the left; while in figures 4 to 9 it is toward the right.

1 The most anterior of the four models seen from the right side. It includes a distance of about two segments. Observe that the roof plate expansion, which covers a large portion of the dorsal surface of the spinal cord, is rather low. It is not known where it begins, or whether there are other outcroppings in front of it as there are behind. It extends caudad some little distance on this model. On the dorsal surface of the cord one dorsal or sensory root is shown, and on the ventral side one ventral root is seen in entirety, being composed of several rootlets. × 50.

2 Dorsal view of the same model shown in figure 1, seen from above. Note that the roof expansion covers a large portion of the spinal cord. At the anterior end it occupies a large central portion, then becomes gradually smaller, and at the same time is confined largely to the left side, after which it gradually increases in size until the posterior end of the model is reached, where it is decidedly wider than at the anterior end, and extends farther over to the right side than to the left. × 50.

3 Ventral view of the cast of the central canal and extension of the same up and out into the roof expansion of the same region of the cord as is shown in figures 1 and 2. It will be seen that the central canal and cavity of the roof plate expansion are connected throughout. In certain places on the right side, shown in white, the roof plate is solid, consisting of ependymal and connective tissue in place of a cavity. For some distance posteriorly on the right side, where the roof expansion is widest, there is no cavity in the roof expansion. The knob-like projections from the right side of the cast of the central canal represent diverticula, and in sections through such a region there would appear to be two central canals. × 50.

ABBREVIATIONS

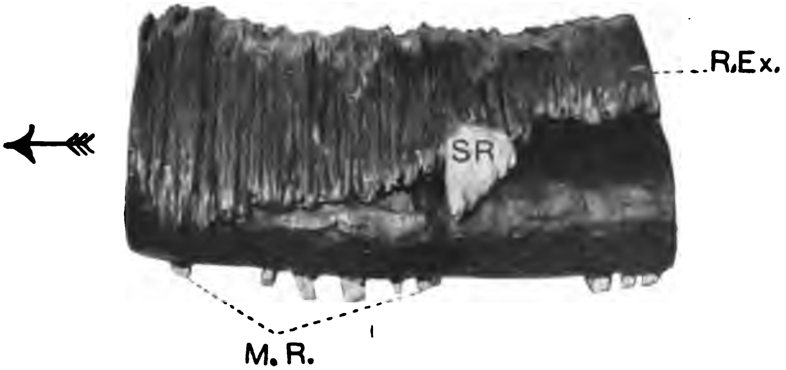
C.C., central canal or cast of the same

C.C.Ex., central canal extension into roof plate expansion or cast of the same

M.R., motor or ventral spinal nerve root

R.Ex., roof plate expansion

S.R., sensory or dorsal spinal nerve root



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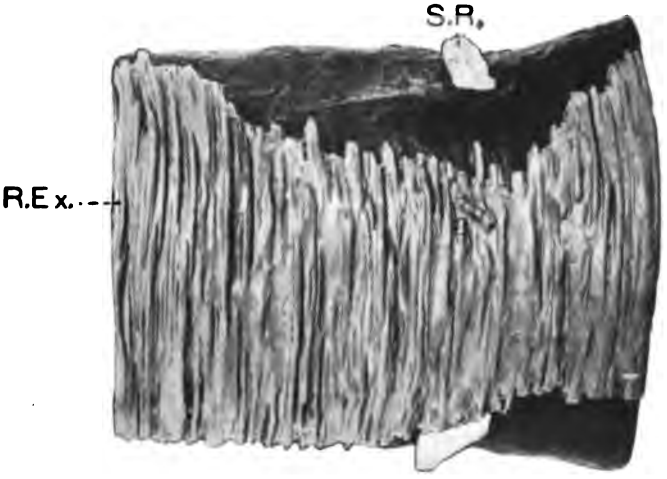

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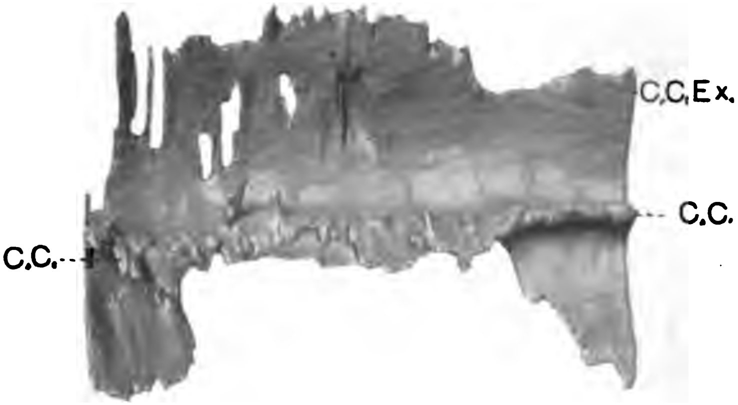

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PLATE 2

EXPLANATION OF FIGURES

4 Lateral view of the left side of the second model of the Polistotrema spinal cord, which should follow figure 1. Observe the continuation of the first roof plate expansion noted in figure 1 and two additional outcroppings of the roof plate (*R. Ex. 2* and *3*). Between these outcroppings the spinal cord is perfectly normal. Three motor and sensory roots are shown in the figure. × 50.

5 Dorsal photograph of the same model as figure 4. The extent and positions of the three roof plate outcroppings previously mentioned in the description of figure 4 are well portrayed here, as are also the sensory roots. × 50.

6 Dorsal view of a cast of the more caudal portion of the central canal and cavity of the first roof plate expansion. This cast differs from the more anterior cast in that there are no connections between the central canal and the cavity of the roof expansion. Evidence from transverse sections favors the view that a connection once existed, which has been cut off in later development. In the two posterior outcroppings of the roof plate there is a connection between the central canal and the cavity of the roof expansion. The light places in the photograph are indicative of places in the roof expansion where it is solid and contains no cavity. They are more numerous and decidedly larger than was shown in the more anterior model (fig. 3). × 50.

Between models 2 and 4 (figs. 4 and 7) comes model 3, the photograph of which has not been included as a figure. It is about equal in length to models 2 and 4, simply connecting the two, without presenting any peculiarities in roof plate expansion, etc.

ABBREVIATIONS

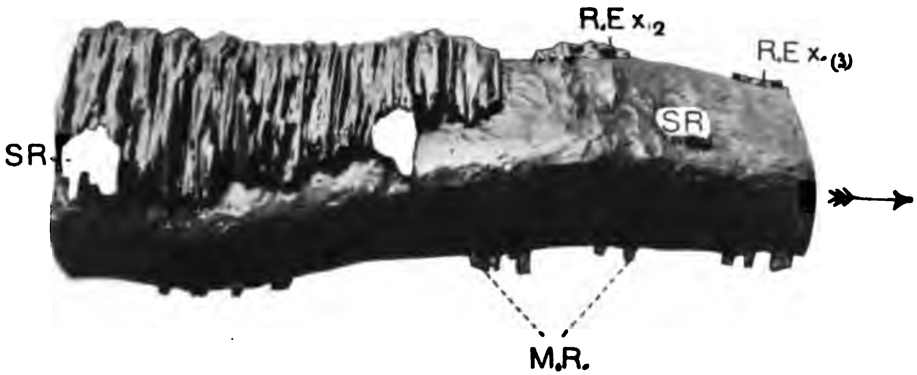
M.R., motor or ventral spinal nerve root

R.Ex., roof plate expansion

R.Ex.(2), second roof plate expansion in the Polistotrema cord

R.Ex.(3), third roof plate expansion in the Polistotrema cord

S.R., sensory or dorsal spinal nerve root



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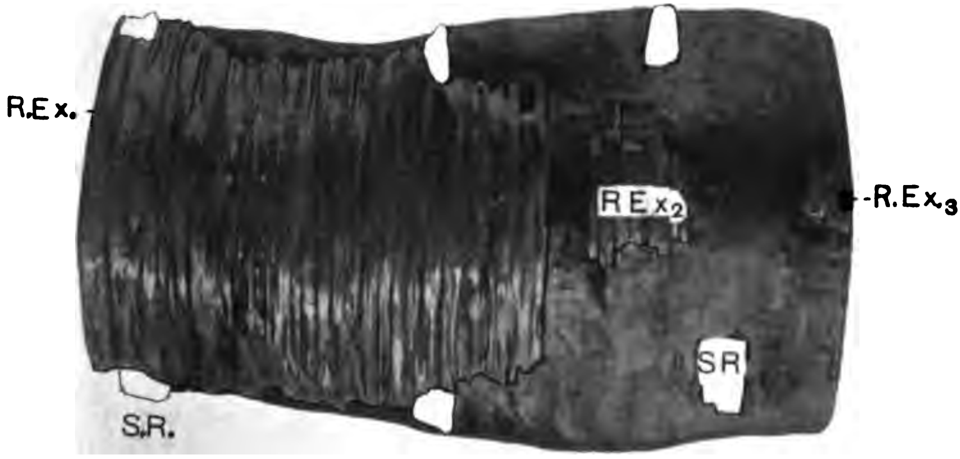

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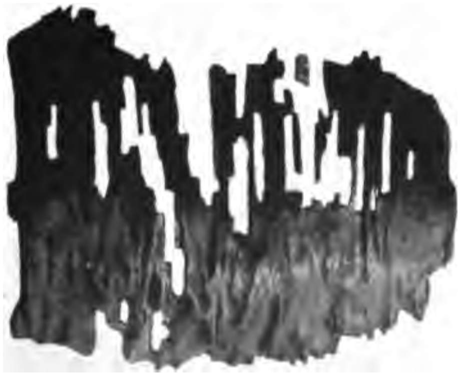

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PLATE 3

EXPLANATION OF FIGURES

7, 8, and 9 represent lateral, dorsal, and ventral photographs of the fourth model, which includes the extreme posterior end of the spinal cord. Note especially the swelling above and below (*S.T.*) caused probably by an abnormal sinus terminalis. From this point caudad two factors occur, which may greatly modify the shape of the spinal cord. First, the notochord gradually decreases in caliber and ends at (*Nc. 1*); second, the spinal cord has not developed a nervous structure, consisting solely of ependyma and round undifferentiated embryonic cells. As a result the spinal cord will be seen to gradually become rounded, and after extending past the notochord it ends in dorsal and ventral processes that become lost in the surrounding connective tissue. The posterior motor roots exhibit a reduction in the number of rootlets, and they approximate each other more closely. The posterior sensory roots become greatly reduced in size, and the last left one has no corresponding roots on the opposite side. × 50.

ABBREVIATIONS

M.R.(1), last motor or ventral spinal nerve root	*S.R.* (1), last sensory or dorsal spinal nerve root
Nc. (1), posterior end of Polistotrema notochord shown in model	*S.T.*, sinus terminalis

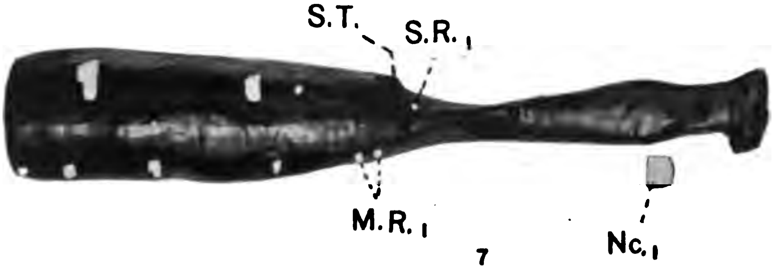
PLATE 4

EXPLANATION OF FIGURES

10 to 23, show 14 transverse sections through the posterior spinal cord region of a 20 cm. Polistotrema (Bdellostoma), the same as was modelled and shown in figures 1 to 9. The outlines were all drawn with the aid of a Leitz-Edinger drawing apparatus, using a magnification of 140 diameters and were reduced one-half in reproduction. They are numbered consecutively from anterior to posterior. Figures 10 to 14 pass through what has been designated as the first roof plate expansion; figures 15 to 17 through the second roof plate expansion; figure 20 through the abnormal sinus terminalis; and figures 21 to 23 at various intervals through the extreme posterior end of the spinal cord, which has failed to develop any nervous structures, and has not to any extent been flattened in its development by the growth of the notochord. The enormous concavity seen on the ventral surface of spinal cord in figures 11 to 14 is to a large extent an artifact due to the preparation of the series. It should be noted that the cavities of the roof plate expansions are full of a fibrillar feltwork, for the most part coagulated cerebro-spinal fluid, that their walls are moderately expanded by it, and their cells are sufficiently granular to suggest a secretary function.

10 is from the most anterior section of this series. The entire neural arch, median dorsal cartilaginous bar, and a portion of the notochord are included in this figure. The so-called first roof plate expansion covers a large area of the central portion of the spinal cord; its cavity is in communication with the central canal. The ependymal walls of both the cavity and central canal are composed of several layers of cells. Motor roots, motor cells, substantia gelatinosa cells, and blood vessels are to be seen in transverse section. × 70.

(*Continued on page 50*)



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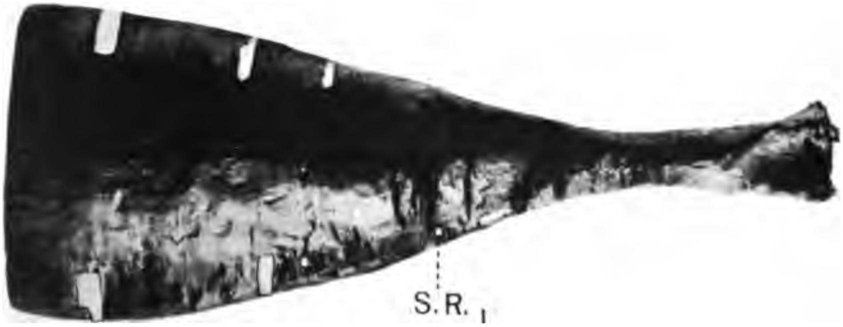

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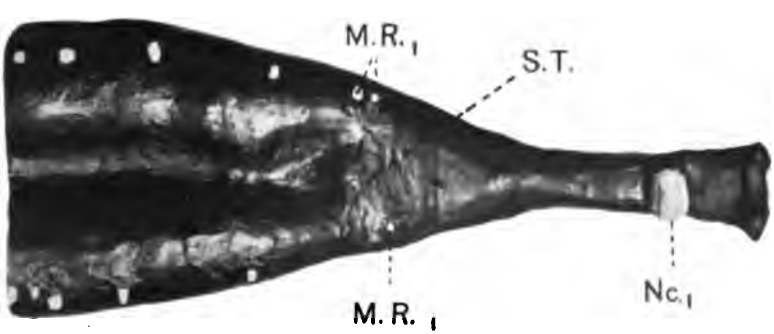

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(Continued from page 48)

11 A transverse section taken 840 microns behind figure 10. It would pass through about the center of the first model (figs. 1 and 2). Note that the roof expansion, while containing an enormous cavity, is confined almost entirely to the right side. It is decidedly suggestive of a chorioid plexus forming cerebro-spinal fluid. A blood vessel lies in a fold in its wall and about this fold there is a great mass of coagulum. Throughout the first roof plate expansion, the cavity, and in many places the central canal itself, is larger than either the third or the fourth ventricle. × 70.

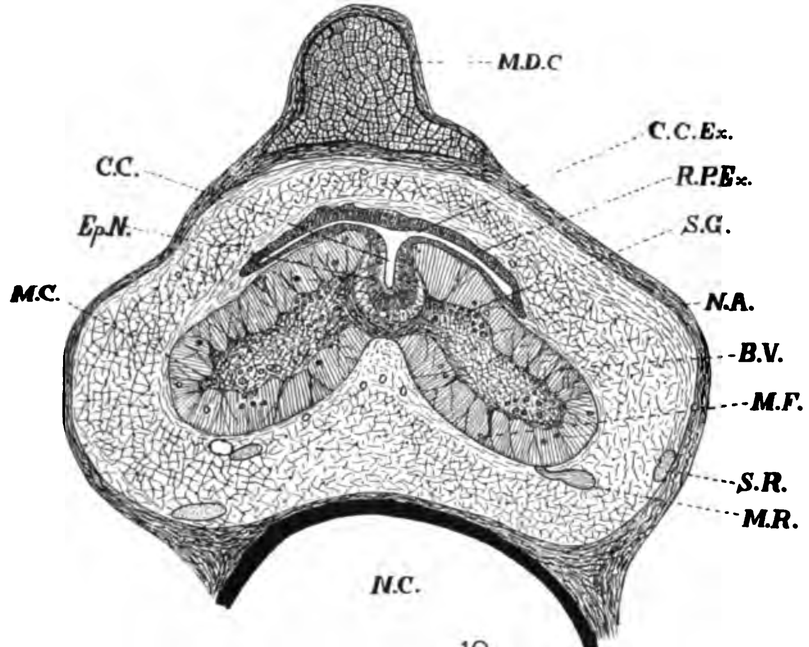
12 From a section 645 microns behind figure 11, and not far from the caudal end of the first model (figs. 1 and 2). At this point the first roof plate expansion and its enclosed cavity attain their greatest width, which cavity is broadly connected with the central canal. × 70.

13 Transverse section 80 microns behind figure 12 and at the very beginning of model 2 (figs. 4 and 5). The dorsal wall of the first roof expansion is very wide and extremely vascular, and the convexity of its walls and the arch of the cavity bear evidence of moderate internal pressure from cerebro-spinal fluid. The wall, while still quite thick, contains a lesser number of ependymal cells, but more connective tissue. Note especially the absence of any direct connection between the cavity of the roof expansion and the central canal. A chain of ependymal cells still connects the two, and may be indicative of a former embryonic connection that has been lost. From this region, caudad, there is no communication between the cavity of the roof expansion and the central canal. That a former connection occurred may be indicated by the fact that at various intervals ependymal cells are scattered between the two. A sensory root can be seen sending its fibers inward toward what is believed to be the substantia gelatinosa. × 70.

13 A, represents a small portion of the first roof plate expansion and its inclosed cavity from a section taken 410 microns behind figure 13. Observe especially the rich blood supply for the dorsal wall and the ease by which diffusion could take place between a blood vessel and the cavity. A large roof plate cell is drawn separately, highly magnified, directly to the left of 13 A. Note the fine granules in cytoplasm, which gives evidence of being secretory. × 125.

ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.C.Ex., central canal extension into roof plate expansion
Ep.N., layer of ependymal nuclei
M.C., motor or effective cells
M.D.C., median dorsal cartilaginous bar
M.F., Müllerian or giant fiber
M.R., motor or ventral spinal nerve root
N.A., membranous neural arch
N.C., notochord
R.P.Ex., roof plate expansion
S.C.F., cerebro-spinal fluid
S.G., substantia gelatinosa
S.R., sensory or dorsal spinal nerve root



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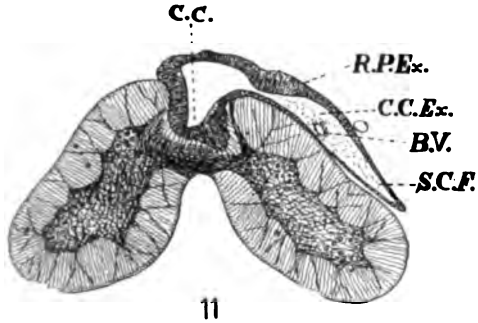

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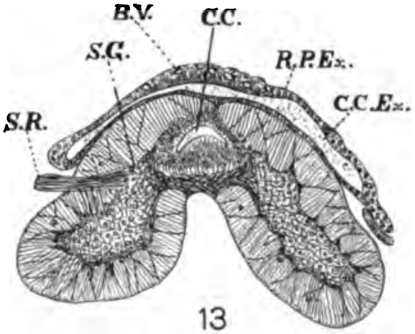

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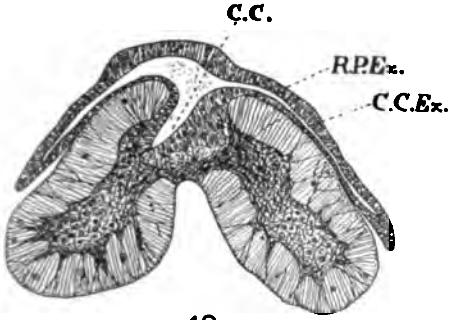

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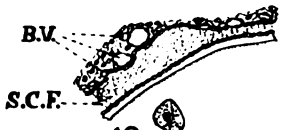

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PLATE 5

EXPLANATION OF FIGURES

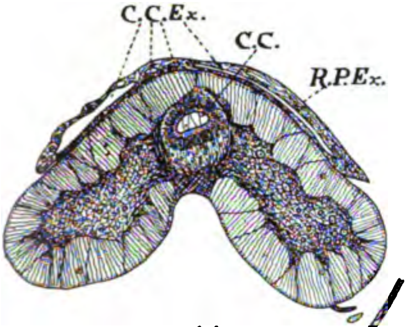
14 From a transverse section taken 19 microns behind figure 13A. It represents a condition found throughout the entire posterior end of the first roof expansion, where a series of cavities are connected with one another. A motor root is seen issuing from the cord. × 70.

15 to 17 Three transverse sections, anterior, middle, and posterior through what has been designated as the second outcropping of the roof plate, which in model 2 (figs. 4 and 5) appears immediately behind the first roof expansion already figured. In figure 15, which is through the anterior end of this roof plate expansion, the outcropping is one of cells only. In figure 16 a distinct cavity containing coagulum is visible, connected below with the central canal. Observe the vascularity of its walls, of the ependyma surrounding the central canal and the diverticula of the same, which doubtless serve as a modified chorioid plexus for producing cerebro-spinal fluid. In figure 17, which is through the posterior end of ths roof expansion, a cavity is still present. Its walls, which are mainly connective tissue, are rich in blood vessels. A few scattered ependymal cells and fibers represent a possible connection with the central canal. A third outcropping of the roof plate has been described, but not figured in transverse section. It is located behind the second expansion, and the fact that no ependymal cells are encountered between the first and the second, and the second and third roof expansions, except immediately surrounding the central canal, favors the view that these separate outcroppings of the roof plate in the adult were never connected in an embryonic state. × 70.

18 and 19 Two sections some distance caudad of figure 17 and some little distance apart. They show the spinal cord to be perfectly normal, but to be gradually tapering down in size. Figure 18 is from that part of the cord represented by model 3. The ependymal area in the center has maintained its original size, the reduction that has occurred in size is to be found in the nervous portion. Not only does the ependymal area broadly divide the spinal cord into two halves, but it has here as elsewhere in a number of places, entirely obliterated the central canal for a space of a few microns. Figure 19 is some distance caudad of figure 18, passing through the sensory nerve roots one segment anterior to the abnormal sinus terminalis (model 4, fig. 9, *S.T.*). The spinal cord will be seen to be fast losing its nervous structure, for no motor cells will be seen in this section or in any further caudad. × 70.

ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.C.Ex., central canal extension into roof plate expansion or cast of the same
Ep.C., ependymal cells
Ep.N., layer of ependymal nuclei
M.D.C., median dorsal cartilaginous bar
M.R., motor or ventral spinal nerve root
M.V.C., median ventral cartilaginous bar
N.A., membranous neural arch
Nc., notochord
R.P.Ex., roof plate expansion
R.P.Ex. (2), second roof plate expansion
S.G., substantia gelatinosa
S.R., sensory or dorsal spinal nerve root



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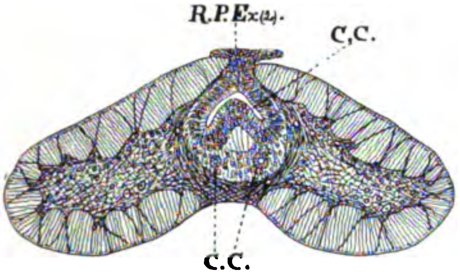

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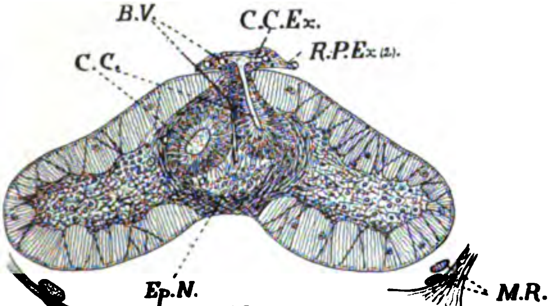

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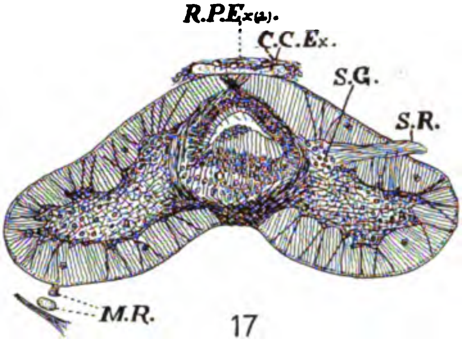

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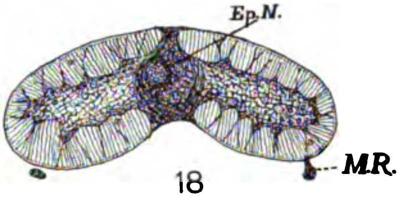

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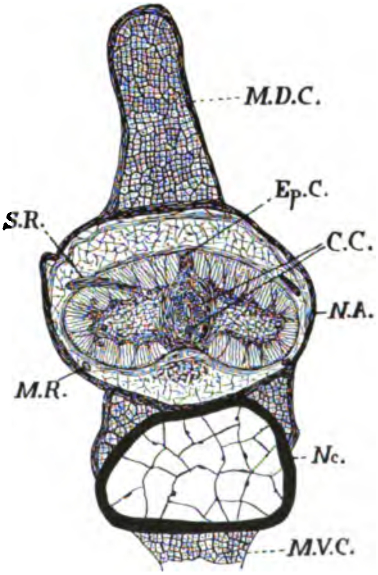

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PLATE 6

EXPLANATION OF FIGURES

20 Through the center of the abnormal sinus terminalis, showing the last pair of sensory roots (not last sensory root) in section. Observe especially the large abnormal sinus together with the rich vascular supply for the tissue immediately surrounding it. Note also the dorsal and ventral swelling caused by this sinus and compare with photograph of the model (figs. 7 and 9). They show every evidence of being expanded by the cerebro-spinal fluid. From this point caudad no nervous structures have developed in the spinal cord. Also from this point caudad the notochord gradually decreases in caliber. × 70.

21 to 23 represent three transverse sections taken through the extreme posterior, non-nervous, end of the spinal cord, which is composed solely of supporting tissue and undifferentiated embryonic cells. Figure 21 is the most cephalic, and passes through the spinal cord a short distance behind the abnormal sinus terminalis (figs. 9 and 20, *S.T.*). The spinal cord is still flattened here, but not indented ventrad, and contains a normal central canal. In figure 22 there has occurred a marked reduction in the size of the notochord. Observe that the spinal cord has not become flattened as it has more anteriorly, where the notochord is massive. With figures 21 and 22 compare figures 59 and 60, which are taken from a similar region of the spinal cord from a 70 mm. Polistotrema embryo. Figure 23 is the last of this series of drawings. It passes through the extreme posterior end of the spinal cord, some 45 microns caudad of the last trace of the notochord. Note the presence of a central canal, and that the cord has separated into several processes, which further on become lost in the surrounding connective tissue. × 70.

PLATE 7

EXPLANATION OF FIGURES

24 to 31 From transverse frontal sections through the medulla and spinal cord of several human embryos, drawn with the aid of an Edinger-Leitz drawing apparatus, and reduced 3 diameters in reproduction.

24 and 25 From transverse sections through the rhombic brain (frontal through the embryo) of a 23 mm. human embryo; figure 25 passes through the V root and posterior end of the cerebellar rudiments (lateral lobes), while figure 24 is from a more anterior section. The roof expansion (chorioid plexus) is shown as a conspicuous black line in these figures. Everywhere within the boundaries of the roof expansion, the cavity is filled not only with coagulated cerebro-spinal fluid, but with embryonic red corpuscles. Whether these entered through venolymphatic openings (C) or are the result of extravasations was not determined. Wherever mesenchyme borders the roof expansion it is very vascular. It is apparent that the roof expansion is under moderate internal pressure. At first glance the roof expansion will show resemblance to the so-called first roof plate expansion of the spinal cord of the 20 cm. Polistotrema, already figured, but its later mode of development was shown to be very different. × 10.

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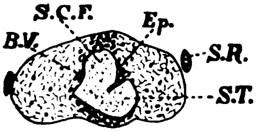

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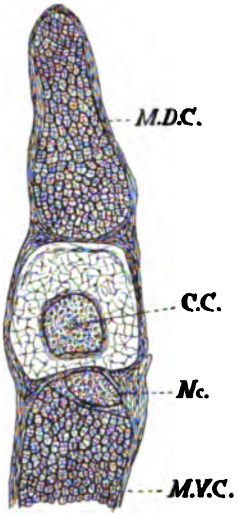

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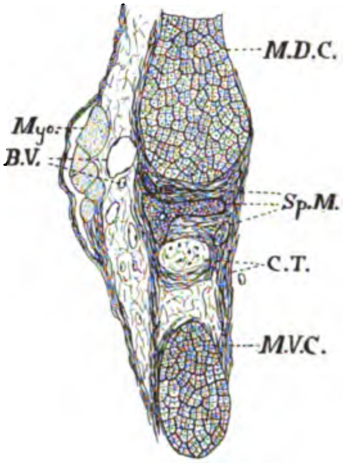

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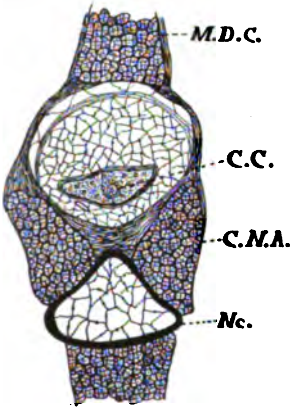

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ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.N.A., cartilaginous neural arch
C.T., white fibrous connective tissue
Ep., ependyma
M.D.C., median dorsal cartilaginous bar
M.V.C., median ventral cartilaginous bar
Myo., myotomes
Nc., notochord
S.C.F., cerebro-spinal fluid.
Sp.M., spinal cord
S.R., sensory or dorsal spinal nerve root
S.T., sinus terminalis

(Continued from page 54)

26 and 27 Represent two transverse sections through the medulla, passing through the posterior end of the fourth ventricle of the same series from which figures 24 and 25 were drawn. Note especially the expansion of the roof plate and compare with the so-called second roof plate expansion of the 20 cm. Polistotrema spinal cord (fig. 16). It is questionable whether the openings (C) are artifacts or not. As was noted previously, the mesenchyme outside the roof plate is very vascular and the roof plate has the appearnce of being under a moderate degree of internal pressure. × 10.

28 A rather oblique frontal section through the medulla of a 15 mm. human embryo (Inst. of Anat., trans. series, H 23). In this region the contour of the rhombic brain is such that the posterior part of the roof expansion of the fourth ventricle is cut transversely; while the more anterior portion, seen below, appears more or less in frontal section. More anterior sections would show the roof plate to be continuous. The posterior end of the fourth ventricle will admit of direct comparison with the second roof expansion of the 20 cm. Polistotrema spinal cord (fig. 16). Had the fourth ventricle been empty, as was the case of the rubber tubing in His' experiments, there would be absolutely no grounds for believing that the anterior portion of the roof plate would be expanded as it is by the appearance of a pontine flexure. It might on the contrary have been folded up within the ventricle. × 16.6.

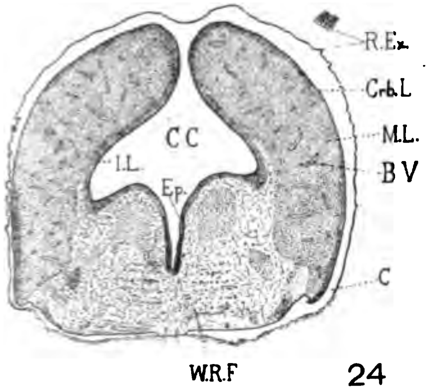
29 A transverse section through the extreme posterior end of the fourth ventricle of the same series as figure 28. There is here a slight roof plate expansion containing no cavity. Compare with figure 15. × 16.6.

30 Transverse section through the thoracic spinal cord, taken from the same series as figure 28. Note that the roof plate consists of ependyma only, while the floor is reinforced by white matter, and even at this late stage if any marked increase in pressure occurred from the cerebro-spinal fluid of this region, an expansion of the roof plate would have been entirely possible. × 16.6.

31 Similar to figure 28, but from an 8 mm. human embryo (Inst. of Anat., series H4). In this plane the posterior end of the fourth ventricle is cut nearly transversely, and is directly comparable with the second roof expansion of the 20 cm. Polistotrema spinal cord (fig. 16). The cavity was full of coagulum, its walls have the appearance of being under moderate internal pressure, and the adjacent mesenchyme is very vascular. × 46.6.

ABBREVIATIONS

B.V., blood vessel
C., an apparent communication between the veno-lymphatics and the fourth ventricle
C.C., central canal
Chcn., chondrocranium
C.P., choroid plexus of the fourth ventricle
Crb.L., lateral lobes of the cerebellum
C.T., white fibrous connective tissue
Ep., ependyma
Ex.Ar., external arcuate fibers
I.L., inner or ependymal layer of nuclei
Mar.L., marginal layer
M.L., mantle layer
Ol., inferior olive
R.Ex., roof plate expansion
R.L., rhombic lip
R.P., roof plate of the central nervous system
S.C., semicircular canals
S.R., sensory or dorsal spinal nerve root
T.S., tractus solitarius
VIII.G., auditory ganglion
V.L.S., veno-lymphatic sinus
V.R., trigeminal root
W.R.F., white reticular formation
XI.R., accessory nerve root



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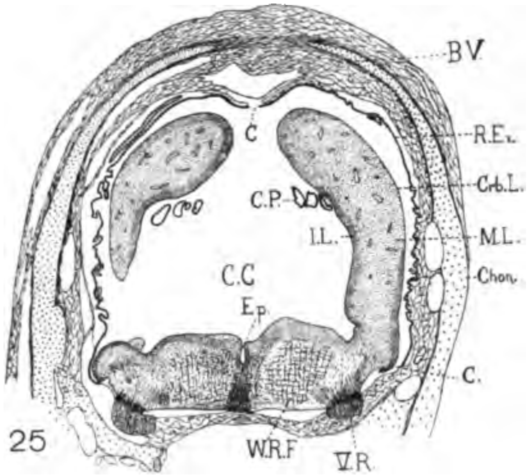

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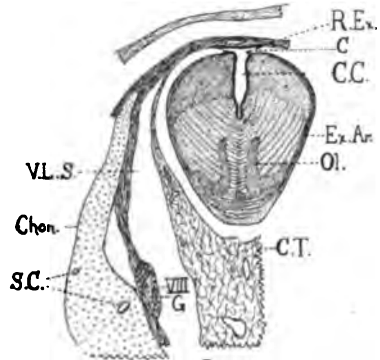

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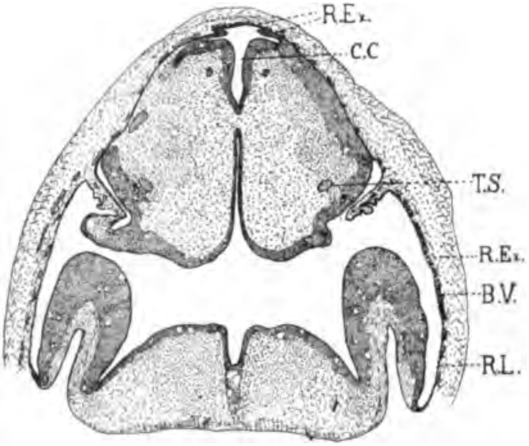

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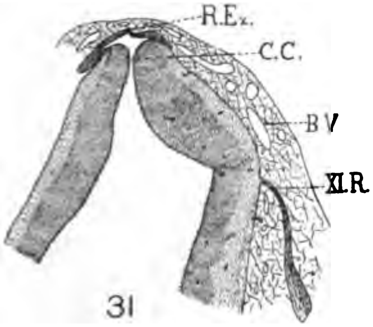

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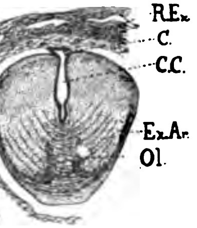

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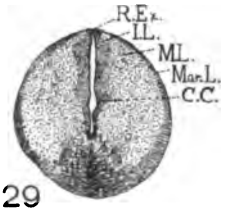

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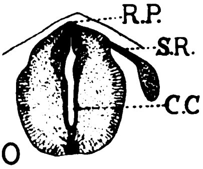

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PLATE 8

EXPLANATION OF FIGURES

32 to 53 A series of transverse sections through the region of the V, VIII, and X ganglia in embryos of Petromyzon of ages varying from 10 to 26 days. It will be seen from these sections that Petromyzon develops an extensive roof expansion without the aid of a pontine flexure, and the cranial and spinal ganglia are well-formed while the central nervous system is a solid cord. All of the figures were drawn with the aid of an Edinger-Leitz drawing apparatus. With figure 54 a magnification of 76.6 diameters was used, while 250 diameters was used for the others. In reproduction they were all reduced one-half.

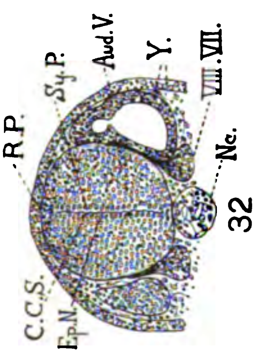
32 Transverse section through the medulla in the region of the auditory vesicle from a 10 day Petromyzon. This is my oldest embryo in which the central nervous system has remained a solid cord. Ordinarily it becomes tubular during the seventh day. This section shows the medulla to consist of a syncytium of protoplasm, consisting of a mass of round nuclei, much yolk, and a few fibers in the marginal layer. The nuclei have migrated a short distance to either side of the median dorso-ventral line. A seam (*C.C.S.*) has appeared here, which marks the position and beginning of the embryonic central canal. The protoplasm bordering the central canal seam is finely granular and may be assuming a secretory function. The acustic ganglia and fibers are shown to be well-differentiated on both sides. × 125.

33 to 35 Three transverse sections passing through the medulla region of another 10 day Petromyzon embryo, in which the central canal has been somewhat retarded in development. These sections pass through the V, VIII, and X ganglia respectively, and with the exception that the central canal furrow or seam (*C.C.S.*) has expanded into small dorsal and ventral cavities (no dorsal cavity has appeared in fig. 35) the general structure of the medulla is about the same as in figure 32. Later these cavities will become the dorsal and ventral expansions of the embryonic central canal of the medulla. The protoplasm in the region of this seam is granular and may be secreting an embryonic cerebrospinal fluid. From these figures it will be seen that the beginning of the central canal occurs at the same time throughout the entire rhombic brain. The anterior portion of the spinal cord, while not figured, contains a central canal furrow in the same stage. × 125.

(*Continued on page 60*)

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
Br.A., branchial arch,
B.V., blood vessel
C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
C.C.S., central canal seam or furrow, in Petromyzon
Ep.N., layer of ependymal nuclei
F.L.P., fused lateral plates of the spinal cord
F.P., floor plate of the central nervous system;
G.C., germinal cell
Mar.L., marginal layer
Mes., mesencephalon
M.L., mantle layer
Myo., myotomes
Nc., notochord
P.B., pineal body
R.P., roof plate of the central nervous system
Sp.M., spinal cord
Sy.P., syncytium of protoplasm
Tel., telencephalon
V.G., Gasserian or semilunar ganglion
VIII.VII., acustico-fascialis ganglion
W.M., white matter
X.G., vagus ganglion (nodosum).
Y., yolk granules



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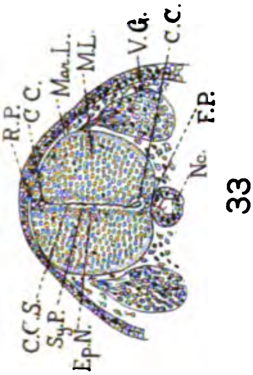

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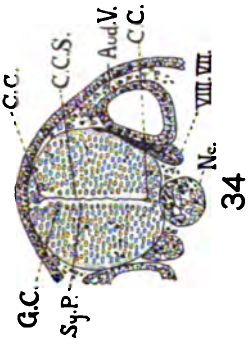

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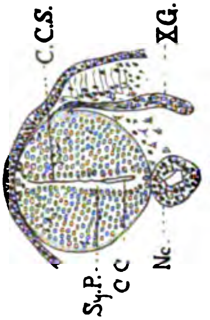

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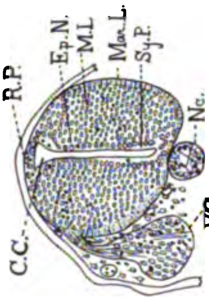

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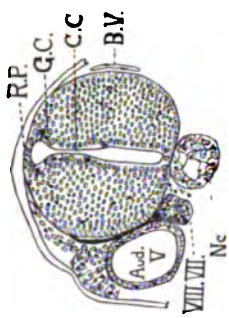

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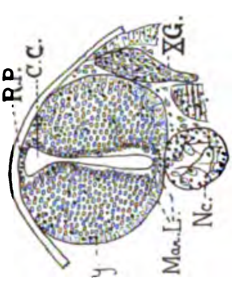

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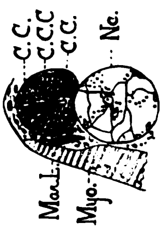

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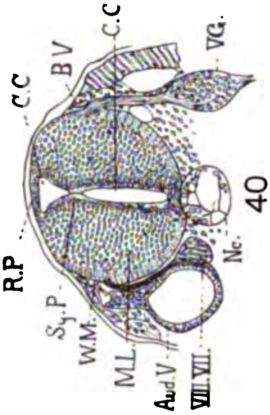

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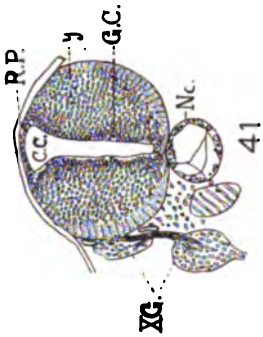

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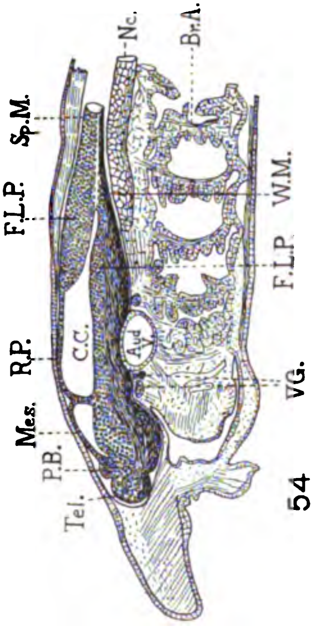

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(*Continued from page 58*)

36 to 38 From a 11 day Petromyzon embryo through the same regions as those shown in figures 33 to 35, and to facilitate comparison were placed directly under them. Considerable progress has occurred everywhere. Note 1) that the central canal seam with its small dorsal and ventral cavities in the 10 mm. series has given place to a typical embryonic central canal, which is much wider at the top and bottom than at the center. The constricted portion of course represents the last place for the protoplasm to give way or to be disintegrated. 2) The floor plate is slightly thicker and less expanded than the roof plate, being reinforced on the outside by white matter and by a rapidly growing notochord below. 3) A few more nerve fibers and nuclei have appeared in the lateral plates. 4) The number of dividing germinal cells has increased while the number of yolk granules remains about the same. 5) The first blood vessels have put in appearance directly outside the meningeal membrane (fig. 37, *B.V.* and nearer the roof plate on the opposite side). × 125.

39 Transverse section through the spinal cord of the same 11 day Petromyzon series as figures 36 to 38, showing the so-called typical embryonic spinal cord. × 125.

40 and 41 Somewhat oblique transverse sections from a 12 day Petromyzon, passing in figure 40, through the V ganglion on one side and the VIII ganglion on the opposite side, and in figure 41 through the X ganglion on one side and a region behind the X ganglion on the opposite side. They can readily be compared with the 11 day series above (figs. 36–38). The lateral plates have apparently increased notably in the number of nerve fibers and nuclei, some of which, however, will have to be attributed to the fact that the sections are cut quite obliquely. Also the numbers of nerve fibers have increased in the floor plate. Throughout, the central canal has increased in width. Of especial interest is a small central mass of protoplasm (*Sy.P.*) in figure 40, which for a space of 50 microns persists as the last remnant of a once solid mass of protoplasm in the center of the medulla. It is obvious at this stage that some factor must have produced sufficient internal pressure to prevent the closing up of the ventricle on account of the rapid increase of cells and fibers in the lateral plates. It is fair to assume that this factor is internal pressure from cerebro-spinal fluid. × 125.

54 of this plate will be described in its proper place, opposite the next plate.

PLATE 9

EXPLANATION OF FIGURES

42 to 44 Three transverse sections through the medulla of an 18 day Petromyzon embryo, passing through the V, VIII, and X ganglia, and for the sake of comparison preserving the same order or arrangement as was used for the earlier embryos. A slight increase in the white matter is to be noted for the lateral and ventral plates over the 12 day series; but little, if any change has taken place in the central canal, unless possibly the central portion has increased slightly in width. Absolutely no further expansion of the roof plate has occurred. Since sections through the medulla of a 14 and 16 day series presented about the same appearance as figures 42 to 44 none were figured. × 125.

45 Transverse section through the cephalic portion of the spinal cord from the same series as figures 42 to 44. Observe especially the beginning of the dorsal closure of the central canal (*C.C.C.*), showing the central canal to consist of dorsal and ventral cavities and a central seam, strongly resembling the stage when it first appeared in the spinal cord. This dorsal closure of the embryonic central canal begins in the anterior portion of the spinal cord much earlier than the corresponding ventral closure of the embryonic central canal in the medulla, occuring in my series of 14 and 15 days. It is obviously caused by the ingrowth of the lateral plates due to the great increase in the number of nerve fibers. × 125.

46 to 48 Three transverse sections through the same region of the medulla of a 20 day Petromyzon as is shown above in figures 42 to 44 for the 18 day embryo. It will be seen that many noticeable changes have taken place, due primarily to a marked increase of nerve fibers in the lateral and ventral plates, and to a slight increase in the number of cells in the lateral plates. The shape of the medulla has become more compressed (flattened out in a dorso-ventral plane). Unquestionably the increase of fibers in the lateral plates has occurred largely in the median and ventral portions. Note the result on the embryonic central canal, which has been completely closed, except for a small dorsal triangular cavity (*C.C.*) the early fourth ventricle. Its roof plate, however, has expanded somewhat. Observe that true ependymal cells are beginning to take on form about the ventricle, and those in the roof plate may soon assume a secretory function, if they are not already active. Also the blood vessels have become more abundant outside of the medulla, especially in the region of the roof plate, which would make infiltration and diffusion through the roof plate into the ventricle easy. × 125.

49 From a transverse section through the anterior portion of the spinal cord. Observe the great increase in fibers in the lateral and ventral plates, and note that the cleft-like embryonic central canal has become entirely closed, but for a small portion, which will remain as the adult central canal. It is obvious that the two portions of the embryonic central canal which persist in the medulla and the spinal cord are the opposite, being the dorsal in the medulla and the ventral in the cord. × 125.

50 to 53 Four transverse sections through the medulla of a 26 day Petromyzon embryo, taken through the V, VIII, and X ganglia, and through a region behind the fourth ventricle where the central canal is passing ventral to assume its characteristic position in the spinal cord. A comparison with the 20 day series above (figs. 46 to 48) will demonstrate a marked increase in the size of the medulla and the fourth ventricle, and a greater expansion and convexity of the

(*Continued on page 62*)

(Continued from page 61)

roof plate, in consequence of which the dorsal tips of the lateral plates are widely separated. There is a marked increase in the number of nerve fibers in the lateral plates, especially in the median and ventral portions. Of prime importance is the great expansion of the fourth ventricle and the roof plate, which apparently in Petromyzon can be explained only from internal factors, the most obvious of which is the mechanical expansion due to an increase in the cerebro-spinal fluid. It will be seen that these forces were sufficiently strong to more than offset the thickening of the lateral plates which would tend to obliterate the dorsal portion of the embryonic central canal as it has the ventral portion. It is apparent that this internal pressure has pushed the lateral wall apart in the dorsal region, where the lateral plates are thinnest and weakest. As was pointed out in the 20 day series the ependymal cells are becoming differentiated and probably have assumed a secretory function. Likewise the increase in the number of blood vessels above the roof plate favors filtration and diffusion into the fourth ventricle. × 125.

54 (See preceding plate.) Median longitudinal section through the head region of a 26 day Petromyzon embryo introduced for a comparison with the transverse sections in figures 50 to 53. Note especially that the marked convexity of the roof plate of the fourth ventricle is suggestive of expansion from an increase of cerebro-spinal fluid. Absolutely no pontine flexure is to be seen, the little convexity that occurs in the floor plate can easily be attributed to an increase in the number of nerve fibers. Observe that the fourth ventricle (*C.C.*) is the remains of the dorsal portion of the original embryonic central canal, while the central canal of the spinal cord is the remains of the ventral portion. The ventral portion of the embryonic central canal of the medulla has been obliterated through the fusion of the ventral portions of the lateral plates. × 38.3.

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
Ep., ependyma
Ep.N., layer of ependymal nuclei
G.C., germinal cell
G.M., white matter
M.L., mantle layer
Myo., myotomes
N.C., nerve cell
R.Ex., roof plate expansion
R.P., roof plate of the central nervous system
V.G., Gasserian or semilunar ganglion
VIII.VII., acustico-fascialis ganglion
W.M., white matter
X.G., vagus ganglion (nodosum).

PLATE 10

EXPLANATION OF FIGURES

55 to 62 Taken from various transverse sections through embryonic and larval Polistotrema and Entosphenus (Pacific coast lamprey). Introduced to show the effect of the developing notochord on the spinal cord in Cyclostomes. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

55 Transverse section through the caudal region of a 20 mm. Polistotrema embryo. It will be seen at this stage that the notochord has produced very little visible effect on the spinal cord. Cyclostome embryos of this stage (compare fig. 39 for Petromyzon) present a nearly cylindrical spinal cord; while that of all other vertebrates is more or less elliptical in cross section, the greater

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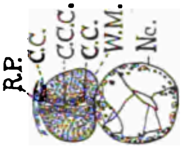

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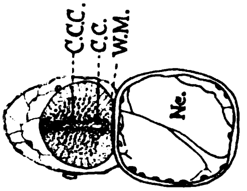

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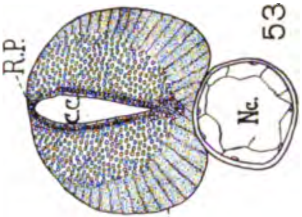

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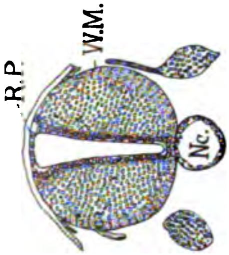

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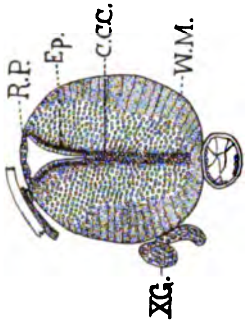

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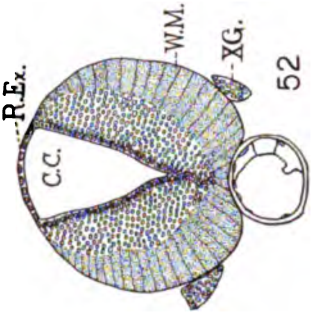

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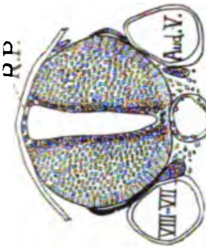

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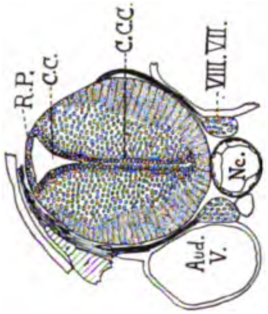

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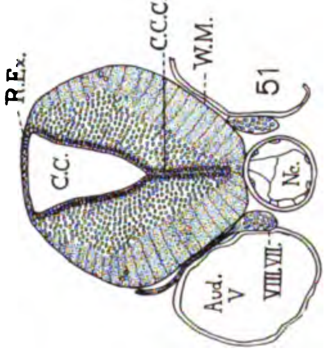

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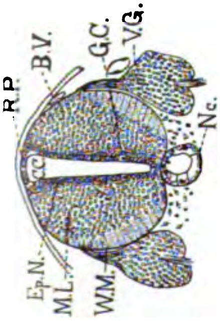

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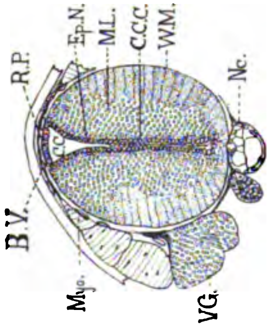

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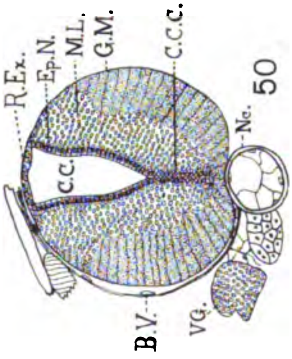

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diameter being dorso-ventral. It should be noted that the spinal cord is enveloped tightly by a meningeal membrane, more or less fused with connective tissue outside that will form the neural arch, which is firmly attached to the notochord below. Immediately above, the mesenchyme is proliferating rapidly and migrating to the center where it will form the median dorsal cartilaginous bar. Little progress has occurred in the formation of the myotomes at the side, and elsewhere there is only loose mesenchyme. × 70.

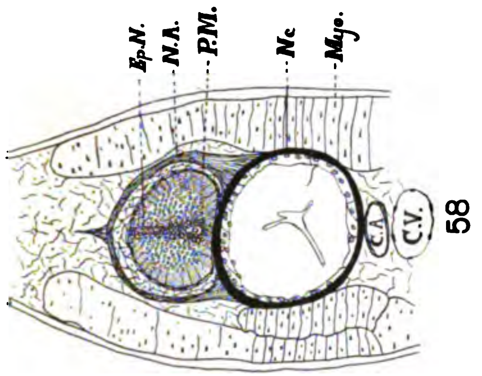
56 Similar transverse section to figure 55, but from a 27 mm. Polistotrema embryo. This slightly later stage shows considerable growth of the notochord and a median indentation on the ventral surface of the spinal cord as the result. Note that the conditions surrounding the development of the notochord previously enumerated under the description of figure 55 are instrumental in assisting the notochord in producing the gradual flattening (depression) of the spinal cord seen in the next figure. × 70.

57 Transverse section of the spinal cord of a 60 mm. Polistotrema embryo from the same region as figure 56. It will be seen that the spinal cord is enclosed in a membranous canal of dense connective tissue, attached below to the notochord and above to the median dorsal cartilaginous bar. Above this there are developing cartilaginous rays surrounded by dense connective tissue. The developing myotomes rest against the neural arches both laterally and dorsally. The notochord has increased greatly in size and, pushing up against the soft spinal cord, produces the depression and ventral indentation of the spinal cord exhibited in this figure. It should be noted that the roof plate is still ependyma and an expansion of the roof plate could take place even in this late stage if the mechanical factors enumerated for the medulla of Petromyzon were operative here. The thickening of the lateral plates has about obliterated the central portion of the embryonic central canal, leaving only the dorsal and ventral portions, in which there is a fibrillar feltwork, probably representing both cerebro-spinal fluid and ependyma cilia. Reissner's fiber is visible in the ventral or permanent central canal. × 70.

58 Transverse section through the tail region of a 20 mm. Entosphenus larva. It will be observed that the spinal cord is further developed than in the 27 mm. Polistotrema embryo (fig. 56). It is apparent that the same factors are involved in flattening the spinal cord as were enumerated for Polistotrema. The notochord has made fully as much growth and the structures surrounding the spinal cord are the same as in Polistotrema, with the exception that instead of a median dorsal cartilage for the attachment of the membranous neural arch there is a membranous neural spine. To some extent this may reduce the dorsal resistance, but on the other hand it may be compensated for by a greater development of the myotomes above the neural arch. × 125.

ABBREVIATIONS

C.A., caudal artery
C.C., central canal
C.H., caudal heart
C.V., caudal vein
D.R., dorsal cartilaginous rays
Ep.N., layer of ependymal nuclei
L.S., lateral veno-lymphatic sinus or anlage of the same
Mar.L., marginal layer
M.D.C., median dorsal cartilaginous bar
M.V.C., median ventral cartilaginous bar
Myo., myotomes
N.A., membranous neural arch
Nc., notochord
P.M., pia mater or meningeal membrane of the younger stages
R.P., roof plate of the central nervous system
Sp.G., spinal ganglion
V.T., ventral veno-lymphatic trunk
W.M., white matter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Myo.
C.A.
C.V.

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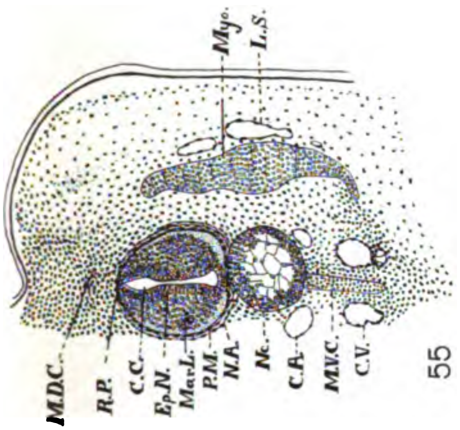
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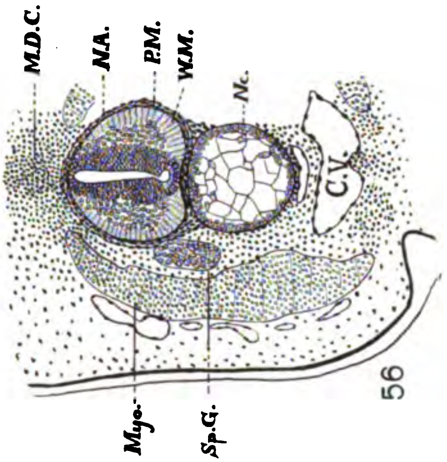
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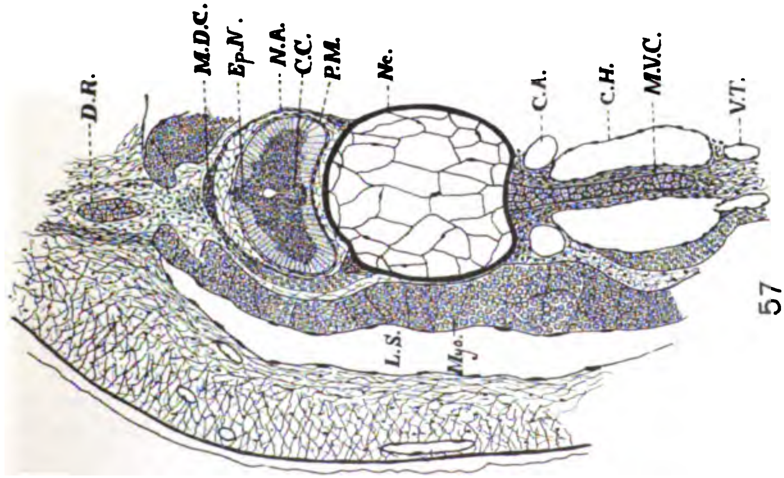
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V.T.
L.S.
Myo.

57

PLATE 11

EXPLANATION OF FIGURES

59 and 60 Two transverse sections only 290 microns apart through the extreme posterior end of the spinal cord of a 70 mm. Polistotrema embryo. In the more anterior section (fig. 59) the spinal cord is depressed, contains developing nervous elements, and the notochord is large proportionately. In the posterior section (fig. 60) the diameter of the notochord is much reduced and only supporting elements appear in the spinal cord. As a result no flattening of the spinal cord has taken place in this region. Compare with figures 21 and 22, which are similar sections through an adult Polistotrema. × 70.

61 and 62 Taken from two transverse sections 480 microns apart, through the medulla oblongata of an adult Polistotrema. In both sections no nervous structures have appeared that were not present in the spinal cord. Note as you pass caudad (figs. 62 to 61) that the medulla becomes flattened ventrally and dorsally in direct proportion to the increase in size of the notochord. This relationship can be shown fully as marked in more anterior sections, and in sections taken from a similar region of larval Petromyzon. × 25.

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal
E.N., undifferentiated embryonic nuclei
Ep., ependyma
Ep.N., layer of ependymal nuclei
L.S., lateral veno-lymphatic sinus or anlage of the same
M.D.C., median dorsal cartilaginous bar
M.F., Müllerian or giant fiber
M.L., mantle layer
M.V.C., median ventral cartilaginous bar
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
P.M., pia mater or meningeal membrane of the younger stages
P.P., parachordal plate
Sp.G., spinal ganglion
W.M., white matter
X.G. in figure 62 should be *Sp.G.*
X.N., vagus nerve

PLATE 12

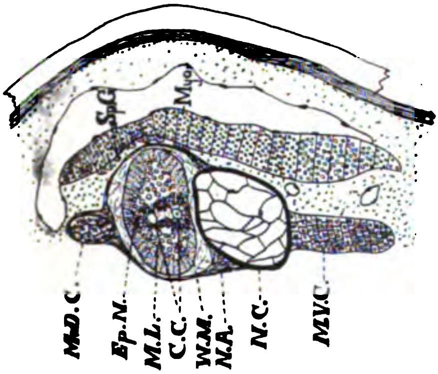
EXPLANATION OF FIGURES

63, represents a diagrammatic reconstruction of the fourth ventricle from an adult Polistotrema series, and the planes from which the transverse sections were drawn for figures 64 to 66 are indicated by lines bearing those figures. Observe how the large fourth ventricle of the embryo has been reduced to a small central canal, having a posterior dilation ($P_4 V$), and how the anterior end breaks up into two or more small longitudinal canals that soon terminate in the sinus mesocœlicus.

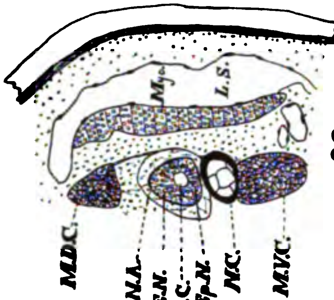
64 and 65, represent transverse sections, taken at different levels of the fourth ventricle of Polistotrema. These sections were drawn with the aid of an Edinger-Leitz drawing apparatus and were reduced one half.

64 From a transverse section through the caudal portion of the mesencephalic lobes (cerebellum of Miss Worthington). Exact plane indicated by line 64 in figure 63. The section passes through the posterior mesocœle or cerebellar ventricle (M''.) and the sinus mesocœlicus (anterior dilation of the fourth ventricle of Miss Worthington), a short distance behind the branching off of the posterior mesocœle. The cavity contains a fibrillar feltwork, which is in part coagulated cerebro-spinal fluid and in part ependymal cilia. The ependyma surrounding the fourth ventricle is rich in blood vessels, which derives its arterial supply from

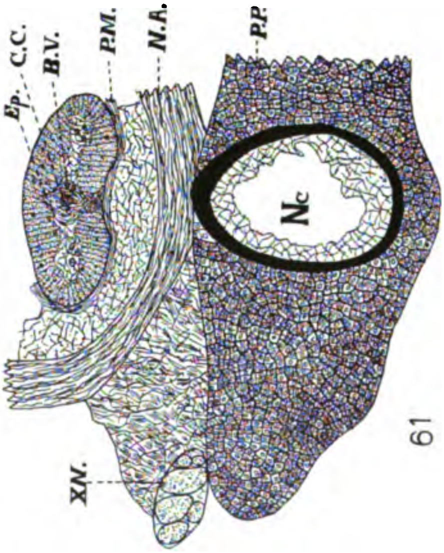
(*Continued on page 68*)


M.D.C.
Ep.N.
M.L.
C.C.
W.M.
N.A.
N.C.
M.V.C.
Sp.G.
Myo.

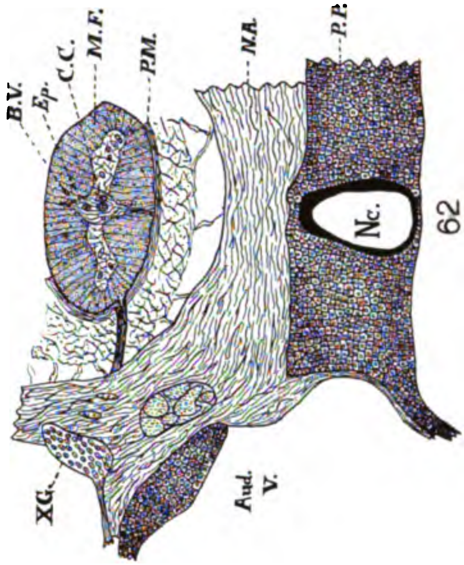
59


M.D.C.
N.A.
E.N.
C.C.
Ep.N.
N.C.
M.V.C.
Myo.
L.S.

60


Ep.
C.C.
B.V.
P.M.
N.A.
P.P.
Nc
X.N.

61


B.V.
Ep.
C.C.
M.F.
P.M.
N.A.
P.P.
Nc.
X.G.
Aud. V.

62

(*Continued from page 66*)

two medulla arteries (*M.A.*). Here as elsewhere, the ependyma surrounding the fourth ventricle doubtless functions as a modified chorioid plexus, discharging cerebro-spinal fluid into the fourth ventricle. It will be seen that the cavity of the fourth ventricle is smaller than the peculiarly modified central canal and roof expansion cavity of the Polistotrema spinal cord portrayed in figures 10 to 13. × 25.

65 A more caudal section through the extreme tip of the posterior lobes of the mesencephalon (cerebellum of Miss Worthington), its exact plane being indicated by line 65 in figure 63. It will be seen that the fourth ventricle of the embryo has in this region of the adult become reduced to three small longitudinal canals ($A_4V.$), which are imbedded in a rather large, dense, and vascular ependymal mass. The most dorsal of these canals contains Reissner's fiber. Here again the ependymal walls are probably functional as a modified chorioiod plexus. Very shortly these canals reunite and continue some little distance caudad as a small central canal, no larger than the central canal of the spinal cord. × 25.

66 Transverse section through the posterior end of the medulla of the same series as figure 64. The exact plane of the section is indicated by line 66 in figure 63. It passes through what has been designated as the posterior dilation of the fourth ventricle (P_4V), which is nothing more than a fair-sized centrally located cavity, the remains of a much larger embryonic fourth ventricle, surrounded by a great mass of vascular ependyma. The center of this cavity contains a fibrillar feltwork (*S.C.F.*) composed largely of coagulated cerebro-spinal fluid and some ependymal cilia. Here as more anteriorly we probably have a modified chorioid plexus, the ependymal walls and their blood vessels secreting and filtering cerebro-spinal fluid into the fourth ventricle. × 25.

ABBREVIATIONS

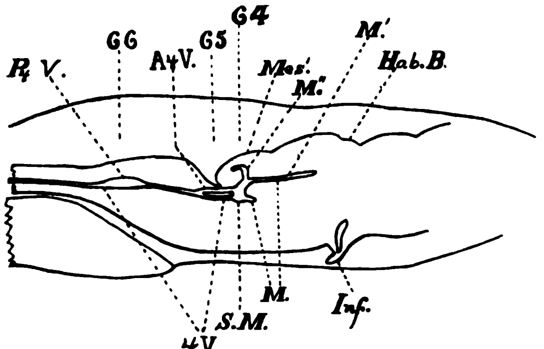
$A_4V.$, anterior fourth ventricle
B.V., blood vessel
Ep., ependyma
Hab. B., habenular body
Inf., infundibulum
M., mesocœle or mesencephalic ventricle
M'., anterior portion of the mesocœle or sub-commissural canal of Nicholls
M''., dorsal portion of the mesocœle or optocoel and posterior portion of the optocoel of Nicholls
M.A., medulla artery
Mes'., posterior lobes of the mesencephalon, cerebellum of Miss Worthington
$P_4V.$, posterior fourth ventricle
S.C.F., cerebro-spinal fluid
S.M., sinus mesocœlicus
V.M.R., motor V root
4V., fourth ventricle
VIII.G., auditory ganglion
X.R., vagus root

PLATE 13

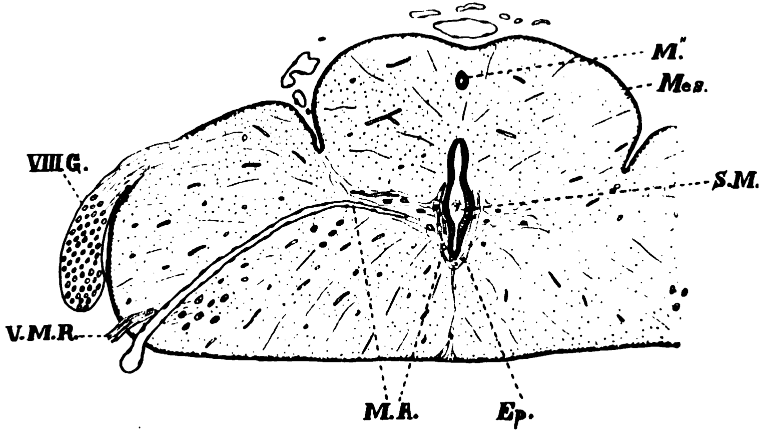
EXPLANATION OF FIGURES

67 to 69 Three transverse sections through the brain region of a 37 mm. Amphioxus. No blood vessels were seen in any of these sections, but the membranous neural canal is surrounded on three sides by enormous veno-lymphatic sinuses, and the structure of the central nervous system is to a considerable extent made up of rather coarse supporting tissue, making infiltration an easy method for nourishing the brain. Drawn with an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

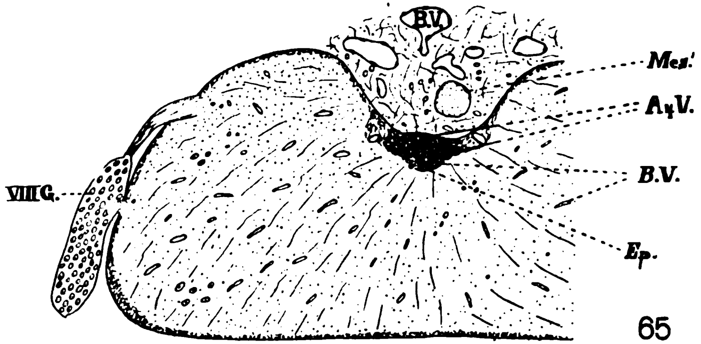
(*Continued on page 70*)


R4 V.
66
A4V.
65
64
Mes.'
M."
M.'
Hab.B.
4V.
S.M.
M.
Inf.

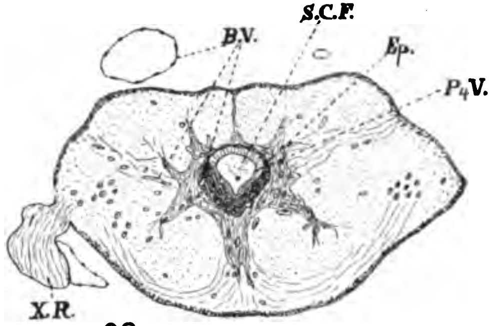
63


M."
Mes.
VIII G.
S.M.
V.M.R.
M.R.
Ep.

64


B.V.
Mes.'
A4V.
VIII G.
B.V.
Ep.

65


S.C.F.
B.V.
Ep.
P4V.
X.R.

66

(*Continued from page 68*)

The most anterior section, figure 67, passes through the anterior ventricle at its highest point, which is a short distance behind the neuropore. This ventricle has no dorsal dilation suggestive of the fourth ventricle. What dilation occurs, is median and ventral. Cilia-like processes from the border of the cells enter the cavity. If the ependymal cells are not secretory it is possible that the cerebro-spinal fluid of Amphioxus does not differ from the serum of the adjacent veno-lymphatic sinuses. If nerve cells occur in this region they are small, and in ordinary preparations indistinguishable from ependymal cells. × 125.

68 60 microns behind figure 67. The large central canal of the embryonic brain has evidently become reduced in this region to a ventral central canal (*C.C.*) and a small dorsal isolated cavity (*V 2*.). This isolated dorsal cavity can not be compared with the fourth ventricle of higher vertebrates. It is rather to be looked upon as a vestigeal structure, which may aid in the infiltration of lymph from the outer veno-lymphatic sinuses. × 125.

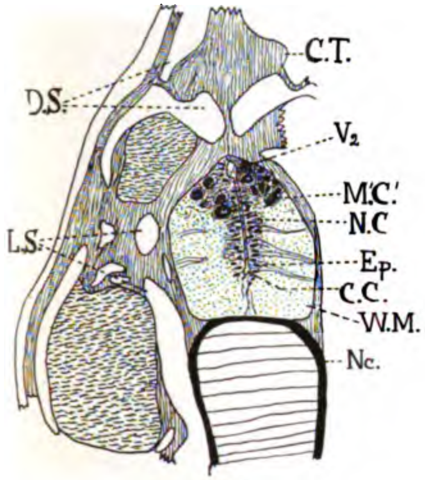
69 Taken from a section 530 microns behind figure 68. It passes through that part of the brain in which there are accumulated a great number of giant cells (*M′.C′.*) in the region of the roof plate. As in figure 68, there is an isolated cavity near the dorsal surface, which was probably a portion of the large embryonic central canal, but which in the adult is separated from the central canal and from the more anterior isolated dorsal cavity by ependyma. It seems best to the writer to regard this and the preceding dorsal cavity as vestigeal structures. × 125.

70 (See next plate.) Transverse section through the anterior spinal cord from the same series as the three previous figures. Observe that the Amphioxus spinal cord is not depressed as is the Cyclostome spinal cord, but is indented ventrally by the notochord. The central canal, which in some places exists as a dorso-ventral cleft, is almost obliterated here by the ingrowth of ependymal tissue. × 125.

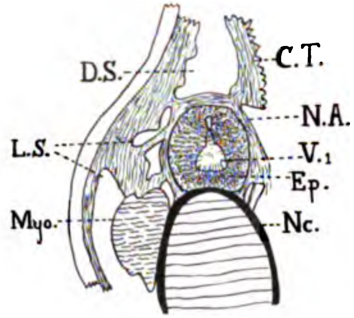
71 Cephalic transverse section through a portion of the spinal cord, meningeal membranes, neural arch, notochord, spinal ganglion,and sensory root of an adult Polistotrema. Observe the depression of the spinal cord, its ventral indentation, the ventral or permanent central canal (*C.C.*), which contains Reissner's fiber, and immediately above, the dorsal portion of the embryonic central canal, which is here more or less filled with ependymal cells and their processes. It will be seen that the gray matter is as much flattened out as is the cord itself, and the ventral horn and motor cells are crowded laterad, while the dorsal horn, substantia gelatinosa (*S.G.*), is apparently median and dorsal. Within the neural arch there is abundant room for a spherical spinal cord. The cord is held in place by the usual meningeal membranes. × 70.

ABBREVIATIONS

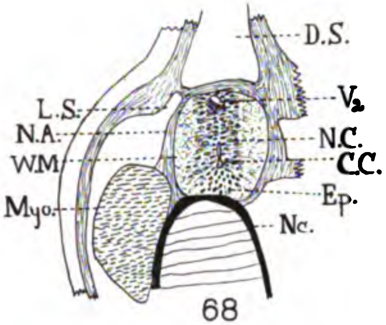
Ar., Arachnoidea
C.C., central canal
C.T., white fibrous connective tissue
D.M., dura mater
D.S., dorsal veno-lymphatic sinus
Ep., ependyma
L.S., lateral veno-lymphatic sinus or anlage of the same
M′.C′., Müllerian or giant cells
M.F., Müllerian or giant fiber
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
Nc., notochord
P.M., pia mater or meningeal membrane of the younger stages
S.G., substantia gelatinosa
Sp.G., spinal ganglion
S.R., sensory or dorsal spinal nerve root
Suba.S., subarachnoid cavities
Subd.S., subdural spaces
W.M., white matter
V.1., anterior ventricle Amphioxus
V.2., vestiges of the embryonic central canal in Amphioxus


C.T.
D.S.
V₂
M.C.'
N.C
L.S.
Ep.
C.C.
W.M.
Nc.

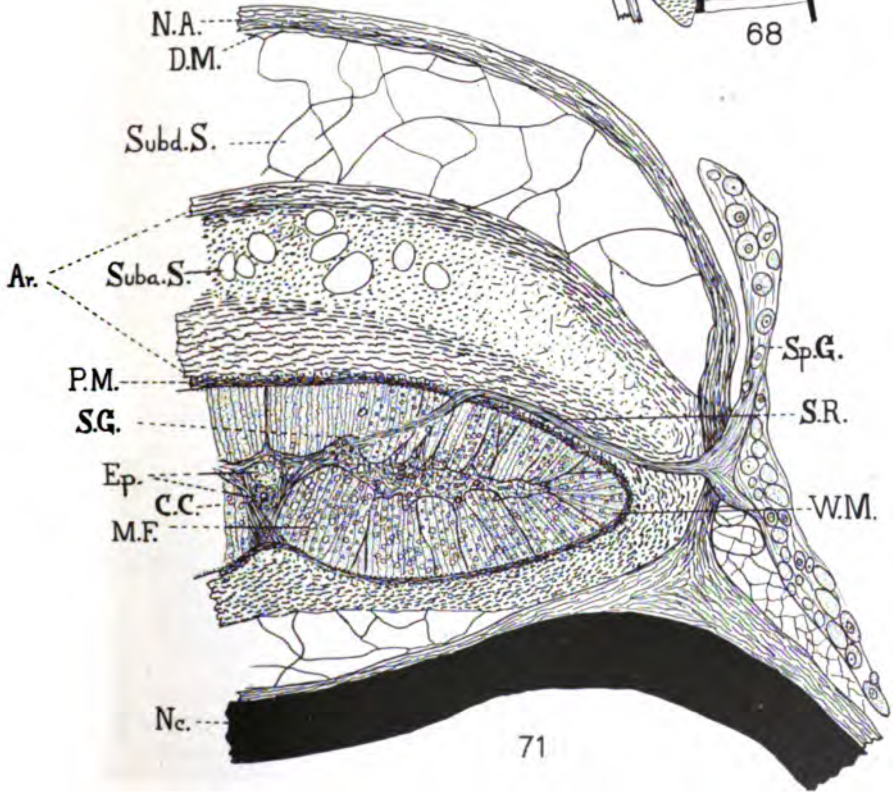
69


D.S.
C.T.
N.A.
L.S.
V.1
Ep.
Myo.
Nc.

67


D.S.
L.S.
V₂
N.A.
N.C.
W.M.
C.C.
Myo.
Ep.
Nc.

68


N.A.
D.M.
Subd.S.
Ar.
Suba.S.
Sp.G.
P.M.
S.G.
S.R.
Ep.
C.C.
W.M.
M.F.
Nc.

71

PLATE 14

EXPLANATION OF FIGURES

72 to 80 represent a number of transverse sections through the medulla of shark, amphibian, and pig embryos for the purpose of demonstrating various stages of roof plate expansion.

72 Rather oblique transverse section through the medulla of a 19 mm. Squalus embryo in the region of the VIII ganglion (from series No. 2 of Professor Scammon's collection). Note the well-formed fourth ventricle, and the broadly expanded and very much stretched roof plate. Its collapsed appearance in this section is doubtless due to fixation or preparation. On account of a great proliferation of cells and nerve fibers the lateral plates have fused ventrally, as in Petromyzon, obliterating that part of the embryonic central canal. Attention should be called to the fact that the medulla roof plate in sharks begins to expand much earlier than it does in Petromyzon. This figure shows a well-expanded roof plate, while the cells in the mantle layer are no more differentiated and there are no more nerve fibers in the marginal layer than appear in a 12 day Petromyzon embryo (fig. 40), where there is no fourth ventricle and no expansion of the roof plate. × 70.

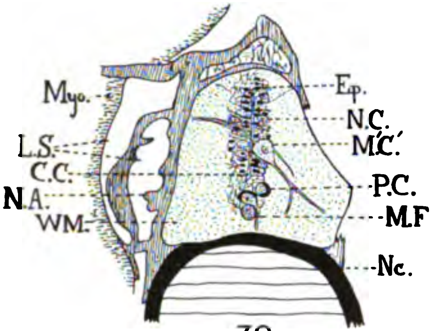
73 Transverse section through the medulla of a 15 mm. Necturus taken through the VIII ganglion. Observe the wide fourth ventricle and the broadly expanded and greatly stretched roof plate, and the coagulated appearance of the cerebro-spinal fluid in the ventricle. Like Squalus, the fourth ventricle begins relatively much earlier than in Petromyzon. It should be noted that no blood vessels have reached the level of the roof plate or entered the medulla; hence the coagulum in the ventricle must be largely a product of secretion. × 39.

74 From a transverse section of a very young Amblystoma embryo in the region of the auditory vesicle (Professor Johnston's series No. 50). Here there has occurred a dorsal and a smaller ventral excavation of the cleft-like central canal. It will be seen that the larger dorsal cavity, the beginning of the fourth ventricle, possesses no thinner roof plate than does the spinal cord (fig. 83). Also in this section (fig. 74) the roof and floor plates are about equally thick. What has taken place dorsally and ventrally throughout the embryonic central canal has been a migration of the cells outward. The fact that in this section of the medulla the roof plate is no thinner than in the section of the spinal cord (fig. 83), taking note that the spinal and cranial ganglia are well-formed, is evidence against the hypothesis, that the greater migration of the neural crest cells of the medulla was the prime cause of the thinning out of the roof plate of the rhombic brain. × 70.

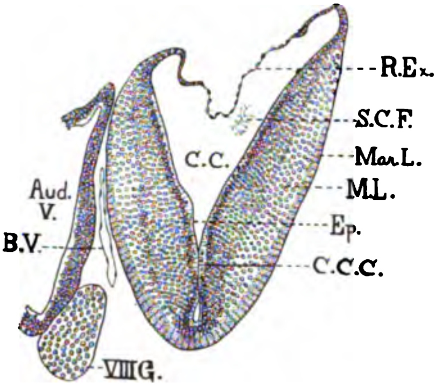
(*Continued on page 74*)

ABBREVIATIONS

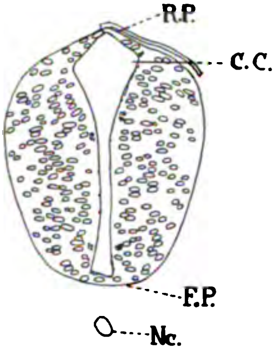
Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal or cast of the same
C.C.C., central canal closure, caused by fusion of lateral plates
Ep., ependyma
Ep.N., layer of ependymal nuclei
F.P., floor plate of the central nervous system
G.C., germinal cell
L.S., lateral veno-lymphatic sinus or anlage of the same
Mar.L., marginal layer
M'.C'., Müllerian or giant cells
M.F., Müllerian or giant fiber
M.L., mantle layer
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
Nc., notochord
P.C., pigmented or eye cells of Amphioxus
R.Ex., roof plate expansion
R.P., roof plate of the central nervous system
S.C.F., cerebro-spinal fluid
V.G., Gasserian or semilunar ganglion
VIII.G., Auditory ganglion
W.M., white matter
4 V., fourth ventricle



70



72



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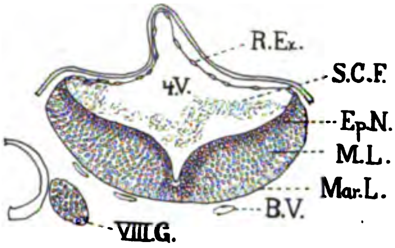
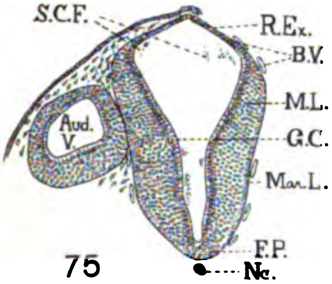
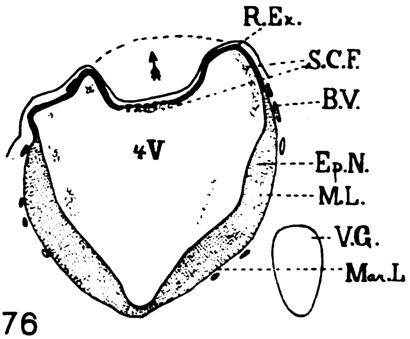


73



75



76

(*Continued from page 72*)

75 to 80 represent five transverse sections through the developing fourth ventricle and roof plate expansion in pig embryos from 5 mm. up to 14 mm. With the exception of figure 75, which is from my collection, the remaining figures are from frontal series belonging to the Institute of Anatomy. That there is a direct relationship between the amount of visible coagulum in the form of a fibrillar feltwork and the expansion of the roof plate is evidenced by the fact that this coagulum does not appear in the early embryos before the roof plate has assumed the appearance of an organ capable of the production of cerebro-spinal fluid (as indicated by vascular supply and granular appearance of the cells). It may be inferred that the earliest non-coagulable cerebro-spinal found in the earliest stages is an embryonic fluid which differs in no way from the ordinary intercellular juices, but that the appearance of coagulum at the time when the roof plate has attained the appearance of a functional chorioid plexus is indicative of a chemical change in the fluid, which if a product of secretion is capable of producing a marked increase of internal pressure in the cerebro-spinal fluid and consequent expansion of the roof plate.

75 From a transverse section through the medulla of a 5 mm. (or less) pig embryo, in the region of the auditory vesicle. It will be seen that the peripheral branches of the intersegmental blood vessels have about reached the roof plate, but no blood vessels have entered the medulla. The protoplasm of the inner margin of the ependymal cells is sufficiently granular to suggest a secretory function. The small amount of coagulum in ventricle is probably the result of secretion, but the cerebro-spinal fluid has probably not exerted much internal pressure. × 70.

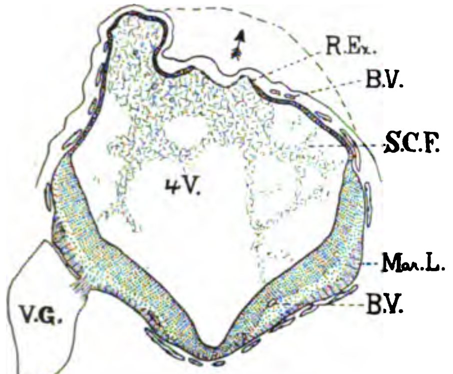
76 Transverse section of a 6 mm. pig medulla through the widest portion of the fourth ventricle, namely, at the level of the V ganglion. This is the only portion of the roof plate to have undergone any stretching of its cells, and this is confined solely to the most centrally located cells. This section shows a considerable increase in the size of the fourth ventricle and expansion of the roof plate, together with some increase in the amount of coagulable cerebro-spinal fluid (*S.C.F.*) and an increase in the number of blood vessels above the roof plate; but no blood vessels have entered the substance of the medulla. At this stage the pontine flexure could not have been a factor in producing the roof expansion. The collapsed appearance of the roof plate at its center is not natural, but rather a result of the preparation of the material. × 39.

PLATE 15

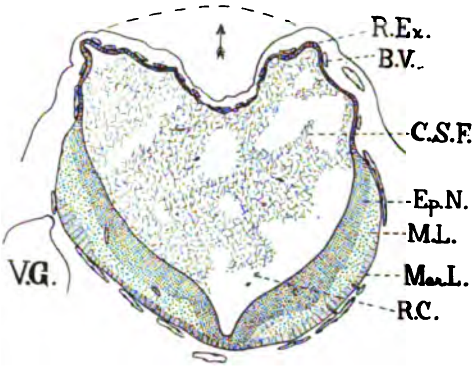
EXPLANATION OF FIGURES

77 Taken from a transverse section through the V ganglion of a 7 mm. pig embryo. Note the increase in the number of blood vessels above the roof plate, which together with the increase in the coagulable cerebro-spinal fluid suggests a functional chorioid plexus. The pontine flexure in this stage is too slight to have any effect on the expansion of the roof plate. It should also be recorded that a few blood vessels have entered the outer surface of the medulla. As in the previous series the sections have suffered a collapse of the roof plate from fixation or later preparation of the material. × 39.

78 From a transverse section of a 10 mm. pig embryo through the region of the V ganglion. The increased vascularity of the mesenchyme above the roof plate together with the enormous amount of coagulated cerebro-spinal fluid (*S.C.F.*) in the ventricle are evidences of the factors which have produced the increased expansion noticed in the roof of the ventricle. Also at this stage the pontine flexure has increased to such an extent that its action on a fourth ventricle full of cerebro-spinal fluid, itself under a moderate pressure, would produce a further expansion of the roof plate. As in the preceding sections the roof plate has suffered a collapse in the preparation of the material. × 39.



77



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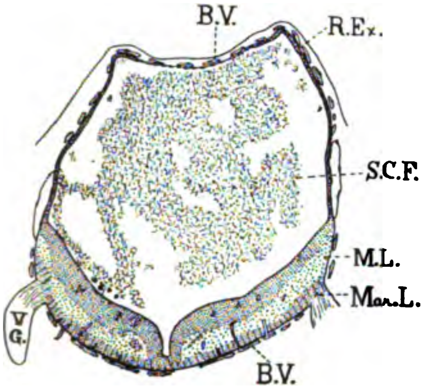

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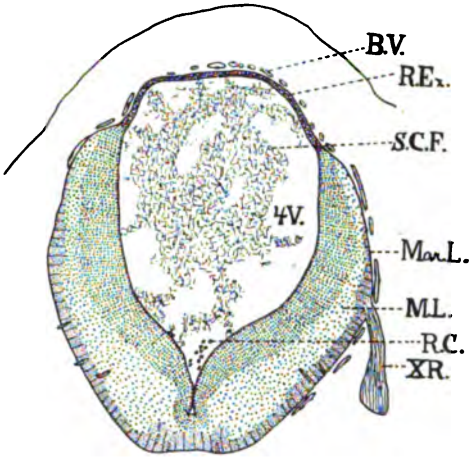


80

79 and 80 Two transverse sections through the anterior and posterior ends of the fourth ventricle from a 14 mm. frontal series of a pig. A more advanced stage in the development of the chorioid plexus together with a more pronounced pontine flexure has produced a much larger fourth ventricle and expanded roof plate than is shown in the previous series (fig. 78). The thickening of the lateral walls of the medulla is taking place as it did in Petromyzon and Squalus, but the greater expansion of the ventricle in the pig (fig. 79) has prevented the walls from fusing ventrally. Nevertheless, the thickening of the ventral portion of the lateral plates would increase the pressure of the cerebro-spinal fluid. × 25 and 39.

ABBREVIATIONS

B.V., blood vessel
C.S.F., cerebro-spinal fluid
Ep.N., layer of ependymal nuclei
Mar.L., marginal layer
M.L., mantle layer
R.C., embryonic red corpuscle
R.Ex., roof plate expansion
S.C.F., cerebro-spinal fluid
V.G., Gasserian or semilunar ganglion
4 V., fourth ventricle
X.R., vagus root

PLATE 16

EXPLANATION OF FIGURES

81 to 87 Represent true transverse sections through what has been termed in the text, the typical embryonic spinal cord, from a number of different vertebrates, all of which have developed a tubular nervous system after the neural fold method. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one half in reproduction.

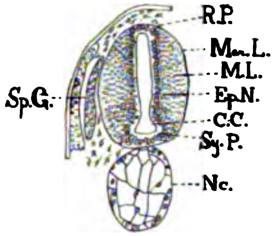
81 From a transverse section through the anterior portion of the spinal cord of a 10 mm. Squalus embryo (Professor Scammon's series No. 16). This so-called typical embryonic spinal cord is decidedly compressed. An earlier stage possessed an elliptical cord with its greatest diameter from side to side. The floor plate is slightly thicker than the roof plate. Each contains a single layer of nuclei. The ventral portion of the cleft-like central canal is expanded into a cavity, which persists as the permanent central canal. A well-formed spinal ganglion is seen to the left. × 125.

82 Transverse section of the spinal cord of a 19 mm. Squalus embryo (from Professor Scammon's series No. 2). Note that the dorsal closure of the lateral plates, due to fiber and cell proliferation, is the same as was figured for Petromyzon. They meet in a seam, leaving dorsal and ventral cavities, of which only the ventral one persists. As in Cyclostomes this method of closure would tend to throw a large part of the embryonic cerebro-spinal fluid into the brain cavities. × 70.

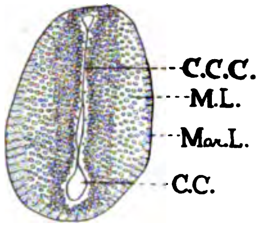
83 and 84 Transverse section through the anterior portion of the spinal cord of an Amblystoma and a turtle embryo. The former (taken from Professor Johnston's series No. 50) is a rather early representative of the so-called typical embryonic stage; while the latter is a rather late representative of this stage. Both cords may be said to be compressed (elliptical, having its greatest diameter dorso-ventral), but only slightly so, when compared with birds and mammals (figs. 85 and 86). As a result, granting an equal proliferation of fibers and cells in the lateral plates, it would be expected that the adult cord in Amblystoma and the turtle would be more depressed, which is found to be the case. × 70 and 125.

85 and 86 From anterior transverse sections of the spinal cord of a 93 hour chick and a 5 mm. pig. Both are good illustrations of the so-called typical embryonic stage, the pig being in a slightly more embryonic state. In these we have the most compressed of all embryonic cords examined, while the adults cord are nearly cylindrical. × 125.

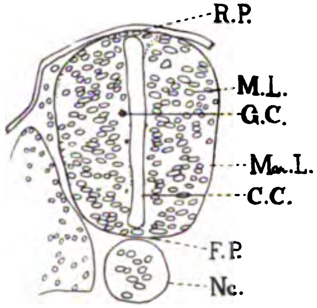
87 Transverse section through the caudal end of the same spinal cord shown in figure 86. Observe spherical appearance which is indicative of an earlier phase in its development. × 125.



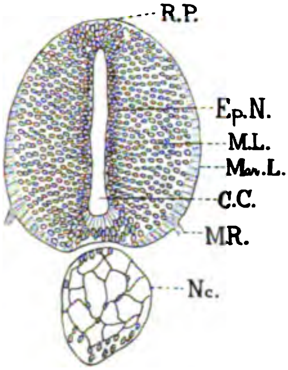
81



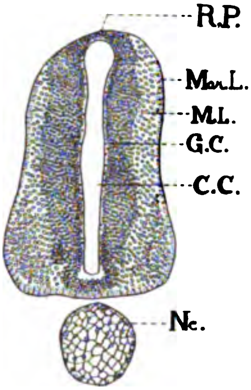
82



83



84



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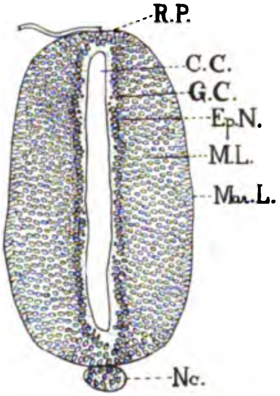

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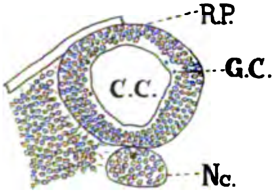

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ABBREVIATIONS

C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
Ep.N., layer of ependymal nuclei
F.P., floor plate of the central nervous system
G.C., germinal cell
Mar.L., marginal layer
M.L., mantle layer
M.R., motor or ventral spinal nerve root
Nc., notochord
R.P., roof plate of the central nervous system
Sp.G., spinal ganglion
Sy.P., syncytium of protoplasm

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THE DEVELOPMENT OF THE LIVER AND PANCREAS IN AMBLYSTOMA PUNCTATUM

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FORTY-SIX FIGURES (FOUR PLATES)

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I. INTRODUCTION

Comparatively little work has been done on the morphology of the biliary and pancreatic duct-systems in vertebrates. The arrangement of these structures has been worked out in the adult forms of a few species but no attempt has been made to correlate these scattered observations or to determine what may be considered the typical arrangement in vertebrates and the major variations which may occur in the various groups of the phylum. The development of these systems is also almost unknown. Although the formation of the anlagen of the liver and pancreas has been investigated in almost every group of vertebrates, the later history of duct systems of these structures has been quite neglected. The two exceptions to this statement are furnished by the work of Corner ('13) who investigated the development of the pancreatic ducts of the pig by means of injection methods and Scammon's study of the biliary system of selachians.[1]

The following study is an attempt to follow in detail the development of these duct-systems in the tailed amphibia, and to point out the embryologic significance of the principal variations which are encountered in the adult and the mechanical influences which are, in part at least, responsible for them. Although we are not as yet in possession of sufficient data to formulate a statement of the typical vertebrate plan of biliary and pancreatic duct-systems, it is hoped that this description of these structures in a representative amphibian may add to the material upon which such a schema must eventually be based.

The material used for this work consisted of embryos of Amblystoma punctatum from 4 mm. to 20 cm. in length. These were sectioned serially in transverse and sagittal planes. Graphic and wax reconstructions were made of the hepatic ducts, gall-bladder, liver and pancreas of different embryos and adults.

It is a pleasure to express my thanks to Dr. Richard E. Scammon for his constant interest and helpful criticisms throughout this study.

A correlation of the embryos employed in this study with those described in the Normal Plates of Necturus maculosus by Eycleshymer and Wilson may be desirable. This correlation is based on a comparison of the digestive system including liver and pancreas, as well as partially on the external form. Probably the greatest difference in the development of the digestive tract between these two forms is in the time of union of the dorsal and ventral pancreatic anlagen which had taken place in most of the 13 mm. Amblystoma embryos which I have observed, and is described in stage 42 (29 mm.) in the Normal Plate series of Necturus. Also the limbs, particularly the caudal ones, appear comparatively later in Amblystoma. Such a table, of course, can be only an approximate comparison.

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[1] The terms used by Scammon ('13) in describing the ducts of Elasmobranchs have been used in this paper.

TABLE 1

Correlation of Amblystoma embryos with the Normal-plate series of Necturus

FIGURES	EMBRYOS	NORMAL-PLATE SERIES	
Figure	Length in mm.	Stage No.	Length in mm.
1	4.5	21	8
2	5	22–23	9
3	7	25	12
4	9	28	15
5	9	29	16
27–30	11	30	17
6	11.5	31	18
21	12.5	34	21
31, 45	13		
18	13	38	25
19	13.5	39	26
8, 39, 40	14	42	29
7A, 9, 41	13.5	43	30
10, 33, 42, 46	15	45	32
11, 20, 43	20	49	39

II. THE DEVELOPMENT OF THE LIVER, HEPATIC DUCTS AND GALL-BLADDER

1. *Literature*

The literature of the development of the great glands of the digestive tract of Amphibia can be conveniently divided into two parts covering two fairly distinct periods: first, the work of the early investigators who determined the position of these glands in the embryo and their relation to the lower germ layer; second, the series of contributions beginning with Goette's large monograph upon the development of Bombinator ('75) and dealing mainly with the detailed developmental anatomy of these organs.

The following table gives a list of the authors, the dates of their publications and the material upon which their work on the development of the liver and pancreas was based.

Steinheim ('20) studied older embryos and observed the attachment to the gut. Rusconi ('26) investigated younger embryos and, as did Reichert ('40) and Vogt ('42), described the ventral growth of the intestine to form the liver. Remak ('55) and v. Bambecke ('68) differed from the above only in the number of lobes formed and noted the close relation of the gall-bladder to the right lobe.

According to Goette ('75), the liver in Bombinator originates as a ventral outpouching of the foregut posterior to the heart. This diverticulum becomes separated from the gut by a gradual cranio-caudal constriction, and the narrow connection which remains forms the common hepatic duct. The outpouching then grows by the production of folds or buds from its sides which form the primary hepatic columns. The lumina remain in these columns although they may be very small. Goette regarded the early anastomoses and formation of the net-like hepatic cylinders as aided by the ingrowth of a capillary network. The gall-bladder develops as an outpouching of the posterior part of the primitive hepatic duct caudal to which the ductus choledochus is formed.

Balfour ('81) made the statement that there is a single ventral diverticulum from the gut which later develops into two secondary branches and so forms the liver.

Shore ('91) in his study on the frog found that the liver takes origin as a ventral lengthening of the gut lumen into the mass of yolk-cells which lies posterior to the heart. The yolk-cells lining this lumen are transformed into hepatic cells and this mass becomes partially separated from the gut. This constriction is aided by the caudal growth of the sinus venosus. Later there is formed at the expense of the yolk-cells and by cell-division a large cell-mass into which the blood-vessels tunnel forming a tubular gland whose columns divide and anastomose producing a network interlacing with that of 'blood-lacunae.'

Marshall ('93) gave a brief account of the development of the liver in the frog in his vertebrate embryology. He described a caudo-ventral projection from the anterior part of the mesen-

TABLE 2

Table of authors and the forms studied

AUTHOR	DATE	MATERIAL
Steinheim	1820	Rana
Rusconi	1826	Rana
Reichert	1840	Rana temporaria Rana esculenta
Rusconi	1854	Salamandra
Vogt	1842	Alytes obstetricans
Remak	1855	Rana temporaria Rana esculenta
Rathke	1861	"Vertebrates"
Bambecke	1868	Pelobates fuscus
Goette	1875	Bombinator igneus
Wiedersheim	1875	Salamandra perspicillata
Balfour	1881	"Amphibia"
Shore	1891	Rana
Goeppert	1891	Salamandra maculata, etc. Bufo vulgaris, etc.
Marshall	1893	Rana
Minot	1893	"Amphibia"
Weysse	1895	Rana temporaria Rana esculenta
Stöhr	1895	Rana temporaria
Hertwig	1896	"Amphibia"
Brachet	1896	Review
Hammar	1897	Rana
Woit	1897	Rana temporaria Triton taeniatus, etc.
Kollmann	1898	"Amphibia"
Gianelli	1899	Triton cristatus
Choronshitzky	1900	Rana temporaria Salamandra maculosa, etc.
Reuter	1900	Alytes obstetricans
Gianelli	1902	Triton
Piper	1902	Review
Weber	1903	Review.
Braun	1906	Alytes obstetricans
Eycleshymer and Wilson	1910	Necturus maculosus
Baumgartner	1914	Amblystoma punctatum

teron. The anterior wall of this depression is thrown into folds, blood-vessels penetrate between these structures and outgrowths from the hypoblast form the hepatic cylinders.

Weysse ('95) found in the frog that the liver-anlage is a dorsoventral cleft extending into the yolk-mass from the gut lumen. A caudal extension of this cleft forms the posterior hepatic duct, while the cranial hepatic duct is formed by a folding of the anterior wall of the hepatic anlage. The yolk-cells are transformed into the true hepatic cells and can be early recognized by the deposit of pigment within them.

Hertwig ('96) and Kollman ('98) gave only short descriptions, stating that in Amphibia the hepatic anlage is a single outpouching from the ventral wall of the duodenum.

Hammar ('97) who worked on the development of the frog's liver, has named the entodermal cell-mass posterior to the heart the 'Leberprominenz.' Into this extends an early lengthening cavity which is continuous with the lumen of the gut. This he termed the 'Leberbucht.' By a cranio-caudal constriction this hepatic anlage is separated from the gut. The cell-mass about the fundus of this anteriorly directed sac develops into trabeculae of the adult organ and the posterior part forms the ductus choledochus. The gall-bladder is developed very early as a diverticulum of the ventral wall of the common bile duct, and by further growth comes to be a pedunculated organ, consisting of a cystic duct and gall-bladder proper. He regarded the origin of the trabeculae as perhaps due partially to the developing capillary network tunnelling into the hepatic cell mass as suggested by Shore.

Choronshitzky ('00) showed the anlage of the liver in the salamander in a figure of a sagittal section of a 9 mm. embryo, in which there is a ventral fold in the wall of the foregut. This fold is lined with yolk-laden cylindrical cells which posteriorly pass gradually over into the polygonal yolk-cells which form a mass projecting into the lumen of the gut. In the anterior ventral wall of the gut is a second slight pouch which later forms the gall-bladder. The two omphalo-mesenteric veins crowd in on either side of the liver outpouching, thereby aid-ing the constriction of the lateral walls of the gut. These veins unite anteriorly and form the ductus venosus. The liver-anlage therefore first grows ventrally and then anteriorly below the horseshoe-shaped union of the omphalo-mesenteric veins and the ductus venosus. A similar sagittal section of a later stage shows the liver at the cranial end of a short ductus hepaticus which is continuous caudally with the ductus choledochus. From the ventral wall of the ductus choledochus there is now a very marked outpouching, the gall-bladder, which is united with the common duct by a short cystic duct. The primitive liver-anlage has thus grown cranialward and become separated from the gut. Choronskitkzy believes this process to be due to the growth and differentiation of the gut. The walls of the primitive liver-anlage have folded and these folds later develop into solid liver-columns. The liver grows around the developing ductus venosus even to its dorsal surface and in so doing produces many folds and columns which grow through the ductus venosus and divide it into sinus-like branches.

Reuter ('00) in his studies on the development of the intestine of the Alytes obstetricans made mention of the early origin of the liver. This develops from the 'Anfangsdarm' division of the midgut. In later embryos the liver develops very rapidly and is divided into three lobes.

Gianelli ('01 and '02) described the hepatic anlage in Triton as developing in two parts, the anterior giving rise to the hepatic tissue proper and the caudal forming the hepatic duct. The gall-bladder arises from a mass of cells belonging to the primitive hepatic outpouching. By the development of the intestinal folds the hepatic duct becomes attached to the dorsal side of the gut.

Weber ('03) stated that the observations made on the development of the liver in the frog and in Triton differ but little. In the latter the intimate relation of the anterior end of the hepatic outpouching and the blood-vessels account for the development of this part into the hepatic tissue proper.

Bates ('04) in a paper on the histology of the digestive tract of Amblystoma has described the hepatic and pancreatic ducts.

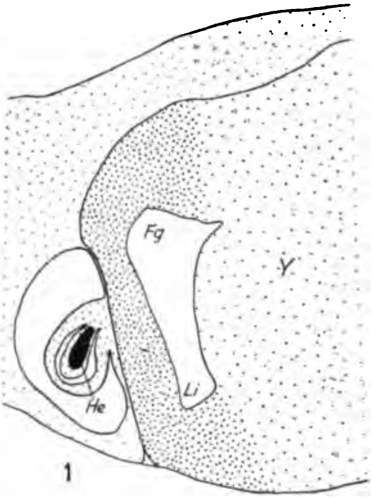
He has described a bile-duct which lies free in the body-cavity for a short distance and then enters the pancreas which lies between the liver and the intestine. Here it is joined by two hepatic ducts and just as this enters the intestine it is joined by two other hepatic ducts.

To summarize briefly, the early investigators described the liver and pancreas as developing at the same time from the ventral wall of the gut, and also considered that they were parts or lobes of the same organ. Remak ('55) first noted that the liver is separate and distinct from the pancreas. Goette first gave a detailed account of the development of the liver in amphibia. Most of the investigators from that time have agreed that the liver begins as a single ventral outpouching of the gut-wall caudal to the heart. The question as to the origin of the gall-bladder, whether from the caudal end of the ductus choledochus or from the wall of the intestine in this region may be, as Piper ('02) stated, one of interpretation rather than one of observation. Whether the hepatic cylinders divide and the blood-capillaries then grow between them, or whether the capillaries grow into the solid hepatic anlage so forming hepatic cylinders seems not to have been definitely determined. Shore's ('91) observations support the latter theory. According to the observations of Weysse and others the yolk-cells are transformed directly into hepatic cells. Very little has been written about the development of the hepatic ducts. The common bile-duct is described as the constricted attachment of the hepatic anlage, or the posterior end of the hepatic outpouching.

2. Early development of the liver

The liver in Amblystoma first appears in embryos about 4.5 mm. in length, which corresponds roughly to no. 21 of Keibel's Normal-plate series. The digestive tract at this stage is quite simple. The pharyngeal cavity is large and extends anteriorly to the oral cavity. Caudally it opens widely into the mesenteron which is composed of a large mass of yolk-cells and extends backward to the proctodaeum. The yolk-mass extends dorsally to the notochord and bulges ventrally.

Posterior to the anlage of the heart a sagittal section shows a ventrally and somewhat caudally directed projection of the gut-lumen, (fig. 1) which extends backward near the dorsal side of the yolk-mass. The anterior wall of the ventrally directed extension of the gut-lumen is lined by yolk-laden columnar cells and its posterior wall is formed by the cells of the large yolk-mass. This cavity is quite wide transversely and is connected to the gut-lumen above by a wide cleft.



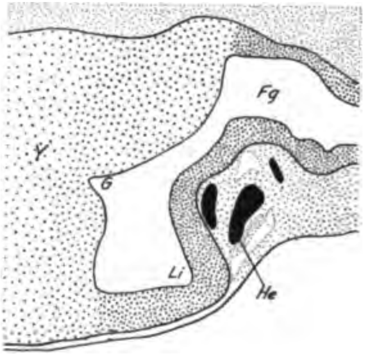


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Fig. 1 Sagittal section of an Amblystoma embryo 4.5 mm. long taken at about the median plane. × 30. *F.g.*, foregut; *He*, heart; *Li*, liver; *Y*, yolk mass.

Fig. 2 Sagittal section of an Amblystoma embryo 5 mm. long, taken to the right of the median line. × 30. *F.g.*, foregut; *G*, caudal extension of gut; *He*, heart; *Li*, liver; *Y*, yolk mass.

Weysse ('95) has described this cavity in frog as a cleft in the ventral mass of yolk-cells, and Hammar ('97) has termed it the 'Leberbucht.' From the study of a slightly more advanced stage Weysse concluded that the caudal and ventral end of this cleft finally formed a caudal hepatic duct. He correlated this with the caudal hepatic duct described in the chick. That the caudal projection does not form a caudal hepatic duct in amphibia seems clear from a study of the later development. The reason for this error was probably, as Hammar has pointed out, that

Weysse did not follow the development beyond a very early stage.

In an embryo approximately 5 mm. (fig. 2) long the anterior wall of this early ventro-caudal projecting cavity has become more prominent. The extension of the gut-lumen into this outpouching is a large cone-shaped cavity somewhat flattened in transection. The columnar epithelial cells lining it are now found farther caudalward than in the preceding stage.

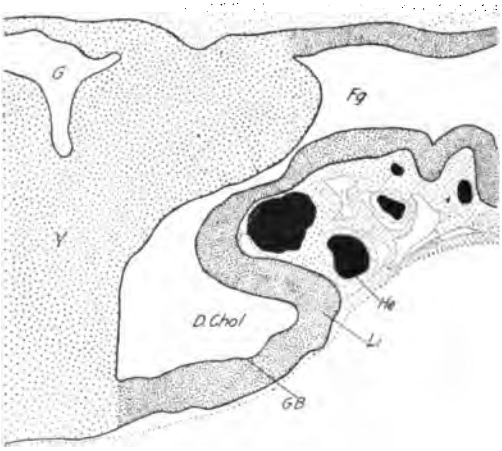


Fig. 3 Sagittal section of an embryo almost 7 mm. long. × 30. *D.chol.*, ductus choledochus; *F.g.*, foregut; *G.* caudal extension of gut; *G.B.*, gall-bladder; *He*, heart; *Li*, liver; *Y*, yolk mass.

In a sagittal section of an embryo 7 mm. long there is shown a more advanced stage of the condition just described. From a comparison of this stage (fig. 3) with the previous one and the one following, it will be seen that the hepatic anlage has become more prominent by a cranio-caudal constriction from the gut. Folds have begun to form on the outer surface of the liver. The cavity of the hepatic diverticulum is widely connected with that of the gut. In the ventral wall there is a slight median depression (*GB*) which is the earliest indication of the gall-bladder. This depression is at the caudal end of the liver-anlage in the region where the primitive ductus choledochus is forming.

The liver of another embryo 7 mm. long appears as an anterior and ventral outpouching of the gut. Figure 36 is of a plastic reconstruction of this region of the archenteron. That the constriction from the gut has proceeded caudally will be apparent by comparison with earlier and later stages. The cavity projecting into the liver-anlage from the lumen of the gut is now much longer, and there are indications of further projections from it on the right side as the lumina of ducts.

Choronshitzky noted this transverse extension of the lumen in the hepatic anlage of the salamander but did not follow its further history. At the posterior end in the median ventral wall is a marked outpouching which is the gall-bladder (*GB*, fig. 36). The opening of this outpouching into the gut is still very wide laterally and shows no differentiation into cystic duct and gall-bladder. The evagination is wide transversely though not extending as far laterally as the liver. In ventral view the gall-bladder appears as a wide transverse outpouching. There is a slight furrow separating it anteriorly and laterally from the liver proper, and a more pronounced one separating it from the caudally placed yolk-mass.

In an embryo approximately 9 mm. in length (fig. 37) the liver is distinctly further advanced than in the preceding one. The caudal constriction from the gut has progressed rapidly (fig. 4). The original anterior convex surface of the liver has become markedly irregular showing numerous depressions or furrows between projecting masses of cells. Greil ('05) figures a model of the liver in a Bombinator embryo 7.5 mm. long with many secondary buds. A network of veins already occupies the spaces between the hepatic buds but Greil only states that it is present. The anteriorly directed cavity has become constricted dorso-ventrally and the division into ducts is more distinct. On the left side (fig. 37) there is a ventral (*vl*) and a dorsal (*dm*) projection of the lumen. On the right side the ventro-lateral extension is prominent. The median ventral evagination (*GB*) has become more pronounced. There is now the beginning of a lateral constriction of this evagination representing the formation of a cystic duct. The anterior lip of the evagination has developed into quite a ridge separating the gall-bladder from the developing hepatic ducts. On the ventral surface the anterior furrow separating liver and gall-bladder from yolk-mass is, as before, the more marked.

According to Shore ('91) in the frog the furrows found in the liver-mass are caused by the 'tunnelling in' of blood vessels. That it is not due only to this is apparent in Amblystoma where sections of this and other embryos show furrows in which there are no blood-vessels (fig. 4). It is important to note that Shore saw no vascular endothelium in these spaces which he regarded as blood-vessels.

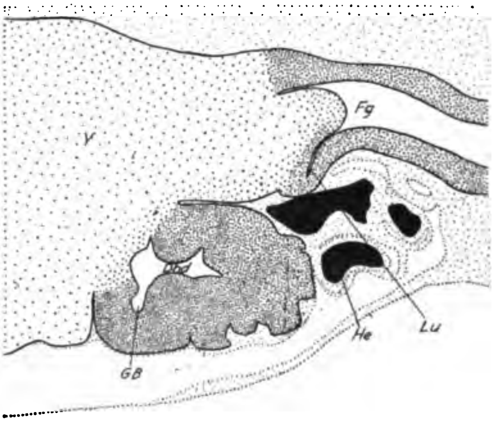
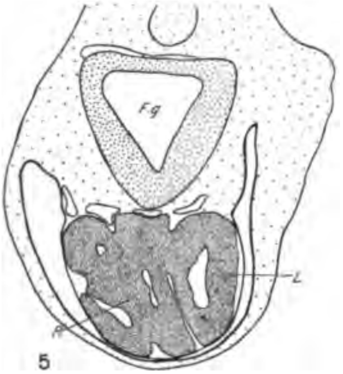


Fig. 4 Sagittal section of an embryo almost 9 mm. long. × 30. *Dhd.*, ductus choledochus; *F.g.*, foregut; *G.B.*, gall-bladder; *He*, heart; *Li.*, liver; *Lu.*, lung; *Y*, yolk mass.

In another embryo of 9 mm. in length the liver in cross section (fig. 5) appears as a large oval mass with an irregular surface showing deep furrows separating the developing ducts. There is also a very marked dorso-ventral furrow separating the liver-mass into two unequal lateral portions of which the left is the smaller. The right portion is marked by two lesser furrows, one ventral, the other lateral.

In 10 mm. embryos a beginning of the network of anastomosing trabeculae can be seen. The development of the sinusoidal capillary circulation in this network has progressed. In the 11 and 12 mm. embryos there is a confusing network of trabeculae and it is difficult to differentiate the main ducts from the hepatic columns. Shore believed that in the frog the tubules were first solid and that later a lumen developed. Goette expressed the opinion that a lumen was present from the earliest formation, though he admitted this was hard to demonstrate. The reason of the difficulty of proving this either way is apparent. However, from a study of sections of Amblystoma it would seem that a lumen is present from the earliest stages.



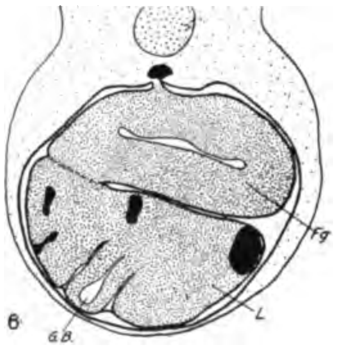
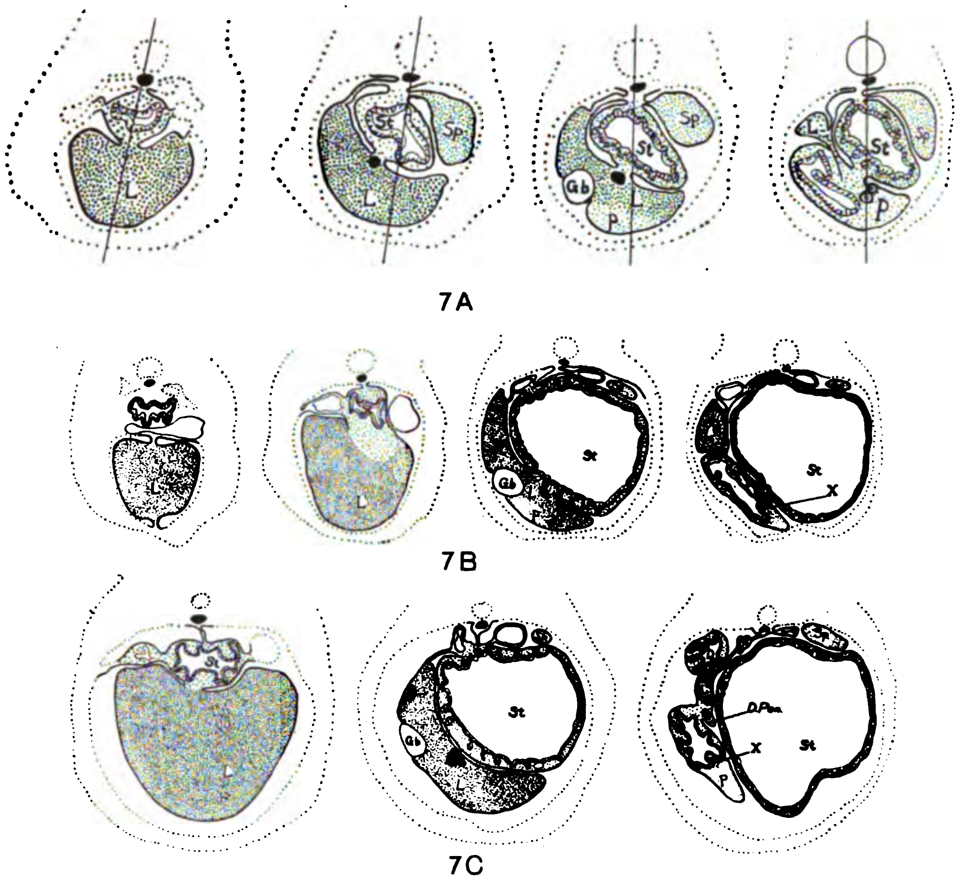


Fig. 5 Transverse section of embryo 9 mm. long. × 30. *F.g.*, foregut; *L*, left portion liver; *R*, right portion liver.

Fig. 6 Transverse section of an embryo 11.5 mm. long. × 30. *F.g.*, foregut; *GB.*, gall-bladder; *L.*, liver.

3. Position of the organ during development

At a stage represented by 11.5 mm. embryos there is a shifting to the right particularly of the caudal end of the liver (fig. 6). Such a shifting of the posterior part of the liver was noted at a later stage in Necturus by Eycleshymer and Wilson ('10) and others. The reason for this lateralward shifting is probably the pressure of the rapidly growing stomach and duodenum which are beginning to take a ventral and sinistral position. It is possible also that the spleen which is now a prominent organ in the left dorsal region of the body cavity has some influence on this



7A

7B

7C

Fig. 7 A series of transverse sections in the region of the liver. A, embryo of 13.5 mm. × 20; B, embryo of 20 mm. × 15; C, embryo of 35 mm. × 10; *G.b.*, gall-bladder; *L*, liver; *P.*, pancreas; *Sp.*, spleen; *St.*, stomach; *x*, ostia of ductus choledochus into gut.

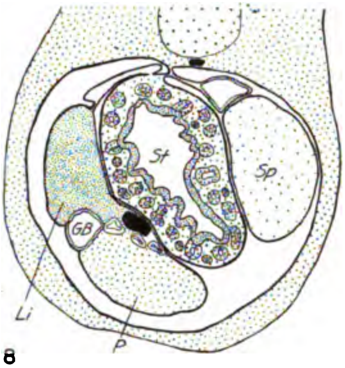
	At level of anterior end of liver	About midway between first and third drawing	Anterior end of gall bladder	Level of attachment of cystic duct to gall bladder	Level of ostium of ductus choledochus
A 13.5..........	“	“	“	Fig. 9 “	“
14 mm..........	—	—	—	Fig. 8 “	—
15 mm..........	—	—	—	Fig. 10 “	—
B 20............	“	“	“	Fig. 11 “	“
C 35............	“	—	“	Fig. 12 “	“

movement. Then, too, the ventral pancreas forms quite a mass in the median ventral region. Figures 7 A, B and C show the lateral and upward shifting of the posterior portion of the liver. The first drawing in each of the series shows a section taken near the anterior end of the liver which here is median and ventral in position and occupies somewhat more than one-half of the area of a circle. The second drawings in figure 7 *A* and *B* show a beginning of a depression on the left side caused largely by the change in shape and position of the stomach and duodenum as mentioned above. Figures 8 to 12 are cross sections of embryos 13.5 to 35 mm. in length showing the position of the liver at the level of the junction of gall-bladder and cystic ducts. Here the lateral and dorsal growth of the liver is marked. A somewhat further shifting is shown in the third drawing of figure 7 *A* and *B* and the second of 7 *C*. These sections were taken near the anterior extremity of the gall-bladder. In all of these the liver is crescentic in transsection and extends upward almost to the level of the dorsal wall of the stomach. The last drawing in figure 7 shows the relation of parts at the level of the opening of the ductus choledochus in the gut. In all cases a small portion of the liver is found dorsal to the duodenum in this region of the embryo. In an embryo 45 mm. long the anterior end of the liver is median and ventral as described above. There is a marked lateral and dorsal growth of the caudal end but in this embryo there is also quite a marked ventral growth which would indicate that from now on the shifting to the right will not be so noticeable, and that there is a growth to the left also.

4. Development of the biliary apparatus

a. Description of the hepatic ducts in the adult. A description of the fully formed biliary apparatus may be of interest before describing the development of the hepatic ducts.

The liver in the adult Amblystoma is a large organ extending fully one-half the length of the abdominal cavity (fig. 13). It has a ventral convex surface conforming to the wall of the abdomen and is divided by an indefinite median line into a right and a left part of which the left is the longer and covers the left ventral surface and a part of the lateral wall of the stomach. The right portion or lobe though somewhat shorter, covers the ven-



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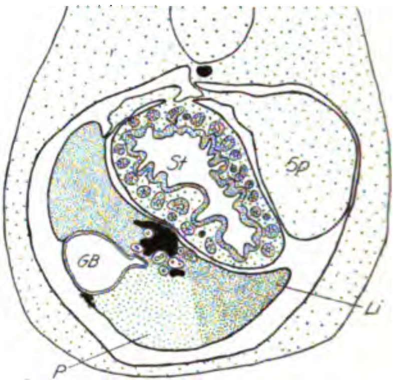

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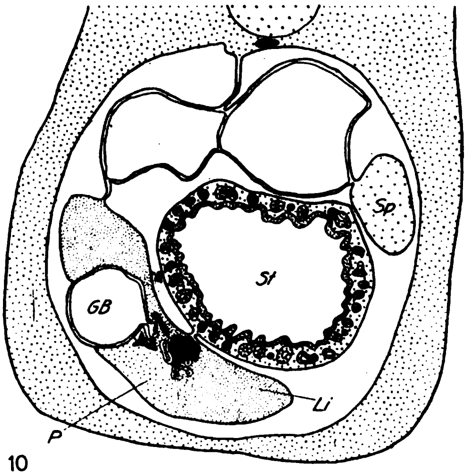

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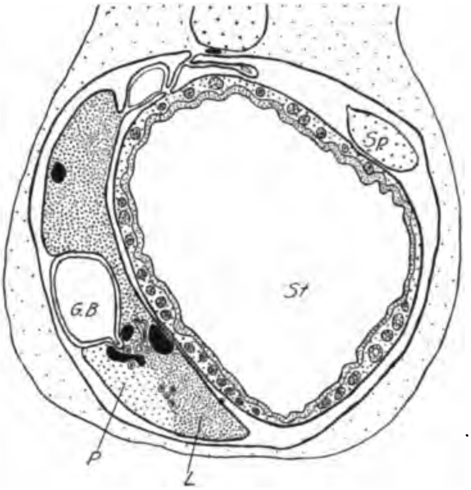
Fig. 8 Transverse sections of an Amblystoma embryo 14 mm. long, taken at level of attachment of cystic duct to the gall-bladder. × 35. *D*, duodenum; *G.B.*, gall-bladder; *Li.*, liver; *P.*, pancreas; *Sp.*, spleen; *St.*, stomach.

Fig. 9 Transverse section of an Amblystoma embryo 13.5 mm. long, taken at the same level as figure 8. × 35. For abbreviations, see figure 8.

Fig. 10 Transverse section of an embryo 15 mm. long, taken at the same level as figure 8. × 35. For abbreviations see figure 8.

tral surface of the stomach to the right of the midline and laterally extends well toward the dorsal wall of the stomach. There



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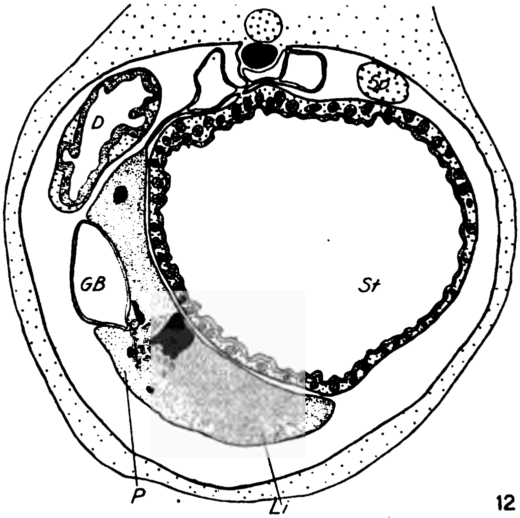

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Fig. 11 Transverse section of an embryo 20 mm. long, taken at the same level as figure 8. × 30. For abbreviations see figure 8.

Fig. 12 Transverse section of an embryo 35 mm. long, taken as in figure 8. × 15. For abbreviations, see figure 8.

are usually one or two lesser indefinite furrows dividing the right lobe into two or three parts. The gall-bladder is embedded in the caudal end of the right lobe some distance from its ventral surface. Only a small part of its rounded fundus appears beyond the hepatic tissue. From the notch in the liver caused by the gall-bladder the one or two lesser furrows of the right lobe extend forward. The gall-bladder is a pear-shaped sac with its larger end extending laterally and somewhat posteriorly. The smaller, medial and ventral end projects forward and connects with the short cystic duct. Only the large blind end of the gall-bladder receives a peritoneal covering, the remainder is embedded in hepatic tissue.

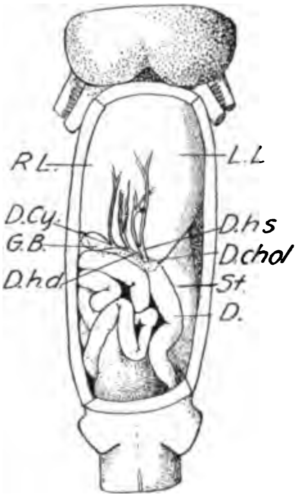

Fig. 13 A dissection of an Amblystoma 12 cm. long. × 1. The ventral abdominal wall has been cut away and the gall bladder and main hepatic ducts dissected out. *D*, duodenum; *D.chol.*, ductus choledochus; *D.cy.*, cystic duct; *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *L.L.*, left lobe liver; *R.L.*, right lobe liver; *St.*, stomach.

There are two main hepatic ducts. These unite to form a common bile-duct of variable length which may be joined by the pancreatic duct just before opening into the gut (fig. 14). Quite often, however, the pancreatic duct opened into the gut immediaately beside the ostium of the common bile-duct. The ductus choledochus is embedded for some distance in the long narrow pancreas lying on the anterior surface of the duodenum and finally empties into the anterior side of the gut near the ventral surface.

The right hepatic duct is divided into lateral and medial rami. The lateral ramus divides into medial and lateral branches. Generally the cystic duct opens into the latter (fig. 14 and 16). However, sometimes the cystic duct is one or

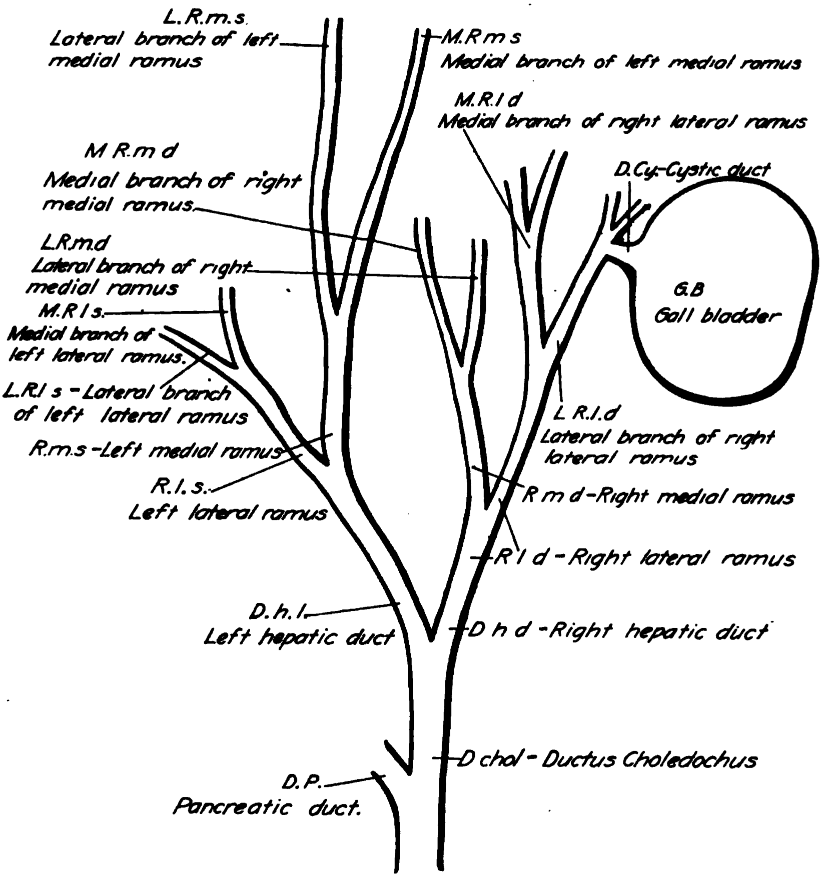
L.R.m.s.
Lateral branch of left medial ramus
M.R m s
Medial branch of left medial ramus
M.R.l d
Medial branch of right lateral ramus
M R.m d
Medial branch of right medial ramus
D.Cy.-Cystic duct
L.R.m.d
Lateral branch of right medial ramus
G.B
Gall bladder
M.R l s.
Medial branch of left lateral ramus.
L.R.l s - Lateral branch of left lateral ramus
L R.l.d
Lateral branch of right lateral ramus
R.m.s -Left medial ramus
R.l.s.
Left lateral ramus
R m d-Right medial ramus
R l d - Right lateral ramus
D.h.l.
Left hepatic duct
D h d -Right hepatic duct
D chol - Ductus Choledochus
D.P.
Pancreatic duct.

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Fig. 14 Diagrammatic drawing of the gall-bladder and hepatic ducts of an Amblystoma.

even two divisions further removed from the common duct, as shown in figure 17 and 44. In a graphic reconstruction of the biliary apparatus of a 7 cm. embryo (fig. 15) the cystic duct joins the right lateral ramus as is shown also in figure 43. The hepatic radicle to which the cystic duct is attached shortly. divides into trabeculae beyond this point. The right medial hepatic ramus divides and subdivides into branches as shown in figure 14. Its branches sometimes anastomose with the branches of the right lateral or left medial ramus (fig. 17).

The left hepatic duct is generally shorter and of slightly smaller diameter than the right one, as well as more ventral in position. It is divided as the latter into lateral and medial rami. The left medial ramus sometimes joins the right medial ramus as shown in figure 16, and this duct then subdivides as a single one. Frequently, however, the left medial ramus runs anteriorly subdividing into smaller branches of which some may anastomose with those of the right medial (fig. 17). The left lateral ramus is shortly divided into two of which the lateral either turns caudally (fig. 44) or sends out branches that go to the posterior portion of the longer left lobe.

b. Development of the ductus choledochus. The ductus choledochus in 9 mm. embryos is still very wide and short. The original caudalward projection from the gut cavity has disappeared and there is only the anteriorly directed common duct. In a model of an embryo 9 mm. long the ductus choledochus is wide transversely but constricted dorso-ventrally (fig. 37 and 38). It is attached at the anterior side of the now ventrally directed gut. At 11 mm. the duodenum has turned ventrally and folded to the right. A very much constricted and short common duct is attached to its superior anterior surface. In a 13 mm. embryo the common duct is attached to the anterior surface of the cranial fold of the duodenum. As before, the duct is small and short, soon dividing into right and left hepatic ducts. The epithelial lining of the duct still contains yolk-granules and except for a quite irregular but prominent lumen is very much like the hepatic ducts. Indeed the difference in the lining cells of this duct and those of the hepatic trabeculae is not great.

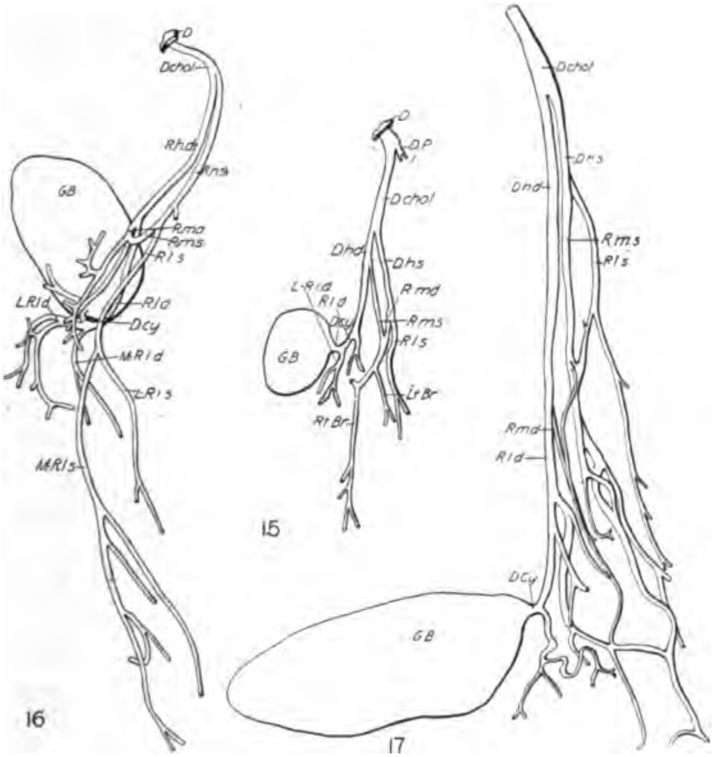

Fig. 15 Graphic reconstruction (lateral view) of an Amblystoma 7 cm. long. × 15. *D.chol.*, ductus choledochus; *D.cy.*, cystic duct; *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *D.P.*, pancreatic duct; *G.B.*, gall-bladder; *L.Br.*, left branch of common ramus; *L.R.l.d.*, lateral branch right lateral ramus; *L.R.l.s.*, lateral branch left lateral ramus; *L.R.m.d.*, lateral branch right medial ramus; *L.R.m.s.*, lateral branch left medial ramus; *M.R.l.d.*, medial branch right lateral ramus; *M.R.l.s.*, medial branch left lateral ramus; *M.R.m.d.*, medial branch right medial ramus; *M.R.m.s.*, medial branch left medial ramus; *R.Br.*, right branch of common ramus; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus.

Fig. 16 Graphic reconstruction (ventral view) of an Amblystoma 10 cm. long. × 15. For abbreviations, see figure 15.

Fig. 17 Graphic reconstruction (lateral view) of an Amblystoma 15 cm. long. × 15. For abbreviations, see figure 15.

In another embryo of approximately 13 mm. length, which is somewhat more advanced, the ductus choledochus is longer and of larger caliber (figs. 18 and 19). It is, however, still attached to the cranial surface of the anterior fold of the duodenum. The epithelium here is now definitely columnar in type, though yolk-granules are still present. In this case the pancreatic duct is attached near the gut to the common duct.[2] In an embryo 13.5 mm. long the ductus choledochus (fig. 7–A) is attached in a fold to the left side of the gut. The duct here is large but shortly divides into the right and left hepatic ducts. The attachment of the duct to the left wall of the gut is to be seen in a less completely developed embryo 14 mm. long. From now on the common duct is attached to the left side of the gut which is faced somewhat cranialward, due to its growth anteriorly and to the right. The length of the common bile-duct before its division varies. In a 35 mm. embryo modelled the common duct is quite long and has a distinct turn shortly before it entered the gut. Here again the pancreatic duct opens into the common duct. There has been a continual change of position of the two ducts from the earliest stage to the fully developed one. In an embryo 13 mm. long a distinct pancreatic duct is seen ventral to the common duct. In the further development with the gradual rotation of the liver to the right there has been a change in position of the common duct until in the 35 mm. embryo it lies to the left of the pancreatic which is the condition found in the adult (fig. 44).

c. *Development of the major hepatic ducts.* The earliest indication of the hepatic ducts was pointed out in the description of the formation of the liver. In a model of an embryo approximately 5 mm. long, as previously stated, the cavity of the early hepatic anlage extends far laterally. On either side the cavity is constricted dorso-ventrally. From the drawings shown by

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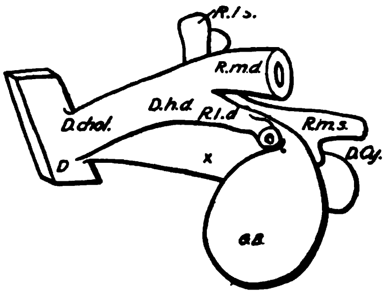
[2] In the further study of the pancreas it was found that this duct was attached by means of a small tubule to the left side of the ventral duct of the pancreas. The epithelial lining resembled that of the gall-bladder, for which this duct was mistaken at first. It might very well be a pancreatic bladder. The pancreatic duct in this embryo was to the right of the enlarged duct.

Choronshitzky it is probable his lateral cylindrical extensions are the early hepatic ducts. In Amblystoma these lateral extensions form only the lateral rami of the hepatic ducts. The medial rami are shown in the model of an embryo about 7 mm.



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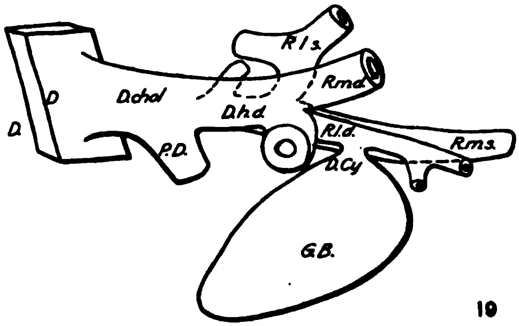

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Fig. 18 Graphic reconstruction (lateral view) of the biliary apparatus of an Amblystoma embryo 13 mm. long. × 100. *D.*, duodenum; *D.chol.*, ductus choledochus; *D.h.d.*, right hepatic duct; *D.cy.*, cystic duct; *G.b.*, gall bladder; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus; *P.D.*, pancreatic duct.

Fig. 19 Graphic reconstruction (lateral view) of the biliary apparatus of an embryo approximately 13.5 mm. long. × 100. For abbreviations see figure 18.

long (fig. 36). On the right side in this model there is a lateral extension of the hepatic lumen. A longitudinal ridge in the floor of this side shows a beginning constriction into lateral and medial rami. The medial ramus is more dorsal in position and appears as a swelling on the outer surface. On the left side there is a wide cavity. On the external surface there is a slight dorso-ventral furrow, an indication of the beginning division into lateral and medial rami.

In an embryo approximately 9 mm. long the right side shows a more marked lateral ramus. The medial still somewhat dorsal ramus is to be seen (fig. 37). Here the left side shows a marked dorso-medial and a ventro-lateral prolongation. The outer surface of both sides of the organ shows many projections, the beginning of tubules from these main rami. The cystic duct though slightly to the right shows more of a constriction from that side. The anterior lip of the cystic evagination also is very prominent.

The rami are formed from the early hepatic ducts by a caudalward constriction and by elongation. Mitotic figures are to be seen at this stage but are more numerous in later ones. As is true of fishes (Scammon '13) there is a relative and actual reduction in the size of these ducts.

In another 9 mm. embryo the development of the ducts is seen to have progressed rapidly (fig. 38). Numerous mitotic figures are to be seen in different sections indicating a rapid growth of the ducts. There are distinct right and left hepatic ducts which show a marked growth. There is a medial longitudinal ridge in the ventral wall of the ductus choledochus indicating a caudalward progressing constriction and division (fig. 38). The cystic duct (*D. cy.*) is distinctly differentiated and attached to the right of the beginning constriction in the common duct. It extends ventrally and somewhat towards the right. The right hepatic duct as seen in figure 38, and in a figure of a model of the cavity of ducts (fig. 20) is divided into a lateral and a dorso-medial ramus. The lateral ramus is further divided into lateral dorsal and medial ventral branches. The left ramus also has medial and lateral divisions.

In embryos from 10 to 12 mm. in length, the trabeculae present a confusing network. The epithelium of both the hepatic ducts and trabeculae are heavily laden with yolk-granules, and that of the ducts is not yet differentiated into a distinct columnar type. However, the right and left hepatic ducts are clear. In an 11 mm. embryo the right duct is distinctly divided into lateral and medial rami. A short cystic duct is attached to the caudal end of the lateral ramus and on its ventral side. In an embryo somewhat less than 13 mm. long the same arrangement of a short common duct and right and left hepatic ducts is present. The right duct is divided into the medial and lateral rami. The cystic duct here projects somewhat to the left and dorsalward connecting as before with the right lateral ramus.

In a graphic reconstruction of a 13 mm. embryo (fig. 18) the right hepatic duct is divided into lateral and dorso-medial rami. The short cystic duct extends upward and opens into the right lateral ramus. A short lateral branch is the only other division of the right lateral ramus. The dorso-medial branch shortly breaks up into trabeculae. The left duct is also divided into rami. The differentiation of hepatic ducts from trabeculae is now clearer as the epithelium of the former is columnar in type.

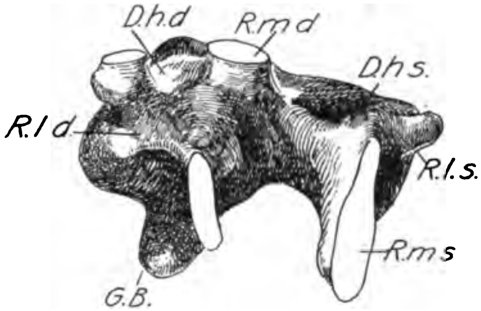


Fig. 20 Anterior view of a reconstruction of the lumina of hepatic ducts and gall-bladder of a 9 mm. embryo. × 100. *D.h.d.*, right hepatic duct; *D.h.s.*, left hepatic duct; *G.b.*, gall bladder; *R.l.d.*, right lateral ramus; *R.l.s.*, left lateral ramus; *R.m.d.*, right medial ramus; *R.m.s.*, left medial ramus.

In figure 19 from an embryo less than 1 mm. longer than the above, the formation of ducts is seen to have continued. The right hepatic duct is divided into lateral and medial rami, each of which is further divided into dorsal and ventral branches. The same holds true in a general way for the left hepatic duct and its divisions.

In the ventral view of the model of a 14 mm. embryo (fig. 39) the relation of pancreatic duct to the common duct is shown. The short thick common duct divides into right and left hepatic ducts (figs. 39 and 40). They lie in almost the same horizontal plane and are of about the same diameter, but the right is the shorter, dividing almost immediately into its lateral and medial rami. In a 13.5 mm. embryo (fig. 41) the right hepatic duct is of larger diameter than the left. In a 15 mm. embryo the common duct is very short (fig. 42). The right and left hepatic ducts here are very long as compared with those in other embryos. The left duct has come to lie in a more ventral plane due to the shifting of the whole posterior part of the liver and gall-bladder to the right. The same is true to a greater extent for the left ducts in the 20 and 35 mm. embryos (figs. 43 and 44). In a 20 mm. embryo the right hepatic duct is the shorter as it is in a 35 mm. embryo. In a 35 mm. stage the left hepatic duct is almost ventral to the right. The same holds true for a 45 mm. embryo. In the adult, however, the left duct is again more lateral to the right, but still somewhat more ventral.

d. Development of the minor hepatic ducts. Right lateral ramus. The right hepatic duct in a 14 mm. stage is divided into lateral and medial rami and the right lateral ramus is subdivided into lateral and medial branches (fig. 39). The short cystic duct is attached to the lateral branch. The medial branch (fig. 40) gives off several tubules in an oblique dorso-ventral plane. In a 13.5 mm. embryo the right lateral ramus is quite ventral to the medial one (fig. 41). As in the earlier stage, it is divided into lateral and medial branches. The cystic duct which is now directed almost horizontally, is attached to the right side of the lateral branch. The anterior portion of the lateral branch anastomoses with a duct from the right medial ramus. In a 15 mm. embryo (fig. 42) the right lateral ramus is shorter than in the preceding specimen. The right hepatic duct is, however, longer so that the cystic duct is attached to the lateral branch farther from the gut. The lateral branch here divides into dorsal and ventral branches. In a 20 mm. embryo the right lateral ramus is very short (fig. 43). In position it is now somewhat dorsal to the right medial ramus. It soon breaks up into dorso-lateral and ventro-medial branches. Both of these branches are very long. At the attachment of the cystic duct to the lateral branch there is a further division of the lateral again into medial and lateral radicles. The medial branch has anastomoses with the right medial hepatic ramus. Its further division is in a dorso-ventral plane. In a 35 mm. embryo the right lateral ramus divides into dorsal and ventral branches (fig. 44). There is another division of the dorsal branch and the cystic duct is attached to the dorsal one of this last division. Frequent anastomoses are formed between the tubules of the dorsal and ventral branches, and between those of the dorsal branch and those from the right medial hepatic ramus, as also of the left medial ramus.

Right medial ramus. The right medial hepatic ramus of a 14 mm. embryo as shown by model is very simple (fig. 39). It joins with the left medial ramus, the further division of this common ramus is into right and left branches. The division of the medial ramus is very short and its lateral and medial branches long. Caudally directed tubules are given off from the lateral branch. The medial branch here is connected with the right lateral ramus. The medial branch divides dorso-ventrally into tubules. In a 15 mm. embryo (fig. 42) the medial hepatic ramus is again very simple. It is short and divides into lateral and medial branches of which the latter is given off almost at right angles and from its anterior surface are given off several tubules. The medial hepatic ramus in a 20 mm. embryo as in a 14 mm. one is joined with the left medial ramus (fig. 43). The resulting common ramus divides into a right dorsal (*R. Br.*) and a left ventral branch (*L. Br.*). From the right dorsal branch, dorso-lateral tubules are given off some of which are directed caudally. In a 35 mm. embryo (fig. 44) the right medial ramus is on the same horizontal plane as the right lateral. Its divisions are also into dorsal and ventral branches. Many anastomoses are found between the tubules of this ramus. Tubules from this ramus join those from the right lateral and from the left medial ramus.

Left medial ramus. The left medial ramus is joined to the right medial in a 14 mm. embryo (fig. 39). In a 13.5 mm. embryo the left medial is long and divides into dorsal and ventral branches (fig. 41). Also in a 15 mm. embryo is the left medial ramus quite long (fig. 42). It divides into medial and lateral branches both of which have dorsal and ventral tubules. The left medial ramus in a 20 mm. embryo (fig. 43) is joined to the right. The left ventral branch of this combined duct divides shortly into dorsal and ventral radicles. In a 35 mm. embryo (fig. 44) the left hepatic ramus is quite long. Its anastomoses with the other rami have been noted. There are also several anastomoses with the left lateral ramus.

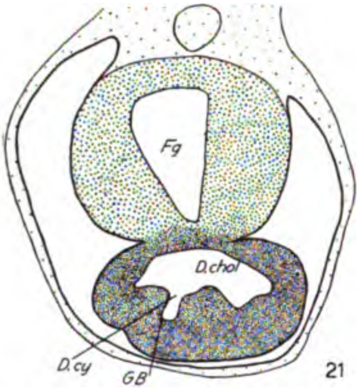
Left lateral ramus. In a 14 mm. embryo the left lateral ramus is very simple, dividing into medial and lateral branches (fig. 40). The left lateral ramus in the next stage shows further development and growth (fig. 41). In a 15 mm. embryo this ramus has lateral branches given off at quite an angle (fig. 42). It is shorter than the left medial ramus and divides into medial and lateral branches, the latter sending tubules far out to the side. The left lateral ramus in a 20 mm. stage is given off nearly at right angles to the left hepatic duct (fig. 43). It divides into dorso-medial and ventro-lateral branches. In this case the lateral branch is the longer. Several tubules go out laterally almost at right angles and from these tubules hepatic columns go posteriorly as well as anteriorly. In a 35 mm. embryo (fig. 44) the left lateral ramus forms quite a network of ducts. The ventral branch makes an arch forward and is then divided into anterior and posterior branches. In an embryo 45 mm. long the main hepatic ducts are more nearly on the same horizontal plane. Of these ducts the left hepatic has extended farther to the left.

e. Development of the gall-bladder and cystic duct. The gall-bladder appears somewhat later than the liver as noted by Hammar ('97). It arises as a median ventral outpouching caudal to or in the posterior end of the hepatic anlage. Choronshitzky has figured the anlage of the bladder in a median, sagittal section. The structure is shown as a slight depression developing from the gut, at the entrance of the common duct, and a definite fold is shown between this and the ventrally extending lumen of the hepatic anlage. Greil ('05) showed the gall-bladder in a Bombinator embryo of 7 mm. length caudal to the hepatic tissue but more closely connected with the liver than with the yolk-mass behind it. In an embryo approximately 7 mm. long, which is undoubtedly an earlier stage in Amblystoma (fig. 3) there is no distinct fold between the gall-bladder and liver-anlage. Only a slight median depression of the floor at the posterior end of the hepatic diverticulum is present. No difference is shown by ordinary stains in the epithelium lining this early cystic evagination and that of the liver. Not until later does the epithelium change into the low cuboidal type characteristic of the adult gall-bladder.

A little later the depression in the floor of the hepatic diverticulum is considerably increased (fig. 36). The position of the gall-bladder with reference to the opening of the hepatic anlage has not changed. In a model of liver and gall-bladder of a 9 mm. embryo (fig. 37) the evagination is quite deep. There is a distinct lateral constriction of the dorsal opening of the gall-bladder and distinct anterior and posterior lips to the evagination, indicating the formation of a cystic duct (fig. 4). There is also a deep furrow anterior to the evagination separating the gall-bladder from the hepatic anlage. The posterior furrow is even more marked. The gall-bladder is, however, still very wide laterally.

In another embryo approximately 9 mm. long the gall-bladder has a long cranio-caudal diameter. The furrow marking off the gall-bladder from the hepatic tissue laterally is distinct. The cystic duct is short and of large diameter and it, as well as the gall-bladder, lies to the right of the midline. The cystic duct projects upward and to the left (fig. 21).

A section of the gall-bladder of an embryo 11.5 mm. long shows there has been a continual shifting to the right (fig. 6). The cystic duct has become longer but is still of wide diameter. It projects more to the left and upward. The gall-bladder, though embedded between hepatic tissue and caudal yolk-mass, is completely separated from both (fig. 22). In figure 23 is shown an increased cranio-caudal diameter, although the transverse is still the greater. The cystic duct here projects more to the left, still somewhat dorsally and slightly backward. The cranio-caudal diameter increases rapidly from now on, and the position of the cystic duct would indicate that there is a more rapid caudal growth. Figure 23 shows the model of a gall-bladder of an embryo almost 14 mm. long. The cystic duct attached near the anterior end, projects to the left and dorsally.



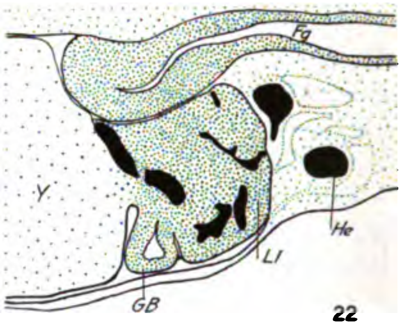


Fig. 21 Transverse section of an Amblystoma embryo 9 mm. long, taken in the region of the gall bladder. × 30. *D.chol.*, ductus choledochus; *F.g.*, foregut; *D.cy.*, cystic duct; *G.b.*, gall-bladder.

Fig. 22 Sagittal section of an embryo 12.5 mm. long. × 30. *F.g.*, foregut; *G.b.*, gall-bladder; *Li.*, liver.

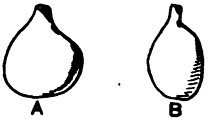


Fig. 23 Drawing of a model of the gall-bladder of an Amblystoma 14 mm. long. *A*, anterior view; *B*, left lateral view. × 40.

In two graphic reconstructions of embryos 13 and 13.5 mm. in length respectively (figs. 18 and 19), the gall-bladder is attached by a short and constricted cystic duct to a radicle of the right hepatic duct. In figure 18 the cystic duct leads from the anterior dorsal end of the gall-bladder to the left, caudally and somewhat dorsally, the gall-bladder being distinctly to the right of the midline. In figure 19 the larger of these two embryos the cystic duct is not quite at the anterior end, but the cranio-caudal length of the gall-bladder is distinctly greater. The general direction of the cystic duct is the same. The gall-bladder is relatively as far caudally here as the one shown in figure 18. From the connection of the cystic duct to the gall-bladder, it appears that there has been a marked growth cranialward.

In an embryo 14 mm. (fig. 39) long the gall-bladder has decidedly increased in its cranio-caudal diameter. In transverse section it is almost circular. The cystic duct is of very small diameter as compared with its earlier size. It projects now somewhat upward but almost directly to the left, due to the increased lateral shifting of the liver and the gall-bladder. In this embryo the cystic duct is attached to the extreme anterior dorsal end of the gall-bladder.

Figure 41 is of a model of a 13.5 mm. embryo. In this the general shape of the gall-bladder is the same as of the one just described, except that there is a slight increase in the vertical diameter (fig. 9). The cystic duct, however, is not attached at the extreme anterior end but to the left upper side. It extends towards the left as before but is now almost horizontal.

In a 15 mm. embryo the attachment of cystic duct to the gall-bladder is further caudalward than the previous one (fig. 42). This seems to mark the limit in its caudal attachment for all sizes examined. It would be difficult to say whether this shifting in attachment of the duct to the gall-bladder were due to a difference in the antero-posterior growth of the gall-bladder or to the rapidity of differentiation and growth of hepatic ducts. The cystic duct in this embryo extends toward the left, but now slightly ventrally, which can be taken as evidence of continued rotation to the right and dorsalward of the entire biliary apparatus (fig. 10).

Marshall ('93) has described the gall-bladder of amphibians developing as a lateral outgrowth from the bile ducts. From its position at this stage one could easily be led to such a conclusion.

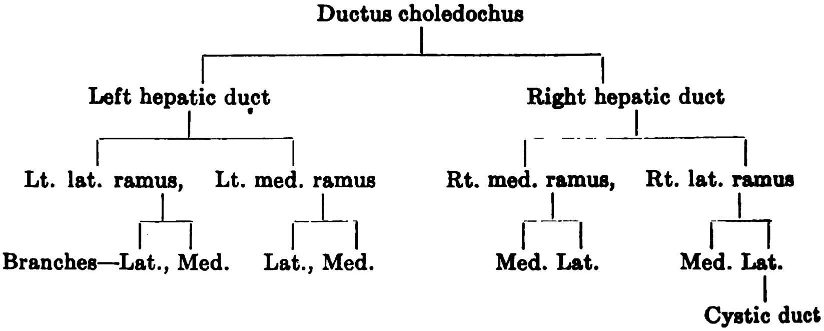
The gall-bladder of a 20 mm. embryo shows a very distinct dorso-ventral increase in diameter (fig. 11). With this there has been a marked cranio-caudal lengthening (fig. 43). The relative size of the gall-bladder is now greater. As before indicated, the cystic duct is here again nearer the anterior end, it extends towards the left and now distinctly ventralward (fig. 11). A right lateral and slightly ventral view of the gall-bladder is shown in figure 43.

In a 35 mm. embryo (fig. 44) the vertical diameter of the gall-bladder has greatly increased. The cystic duct is now in the left anterior ventral end extending ventrally and to the left. In a 45 mm. embryo the gall-bladder has the same general shape as in the preceding, and the cystic duct has not changed in position (fig. 12).

In a graphic reconstruction of the biliary apparatus of a 10 cm. Amblystoma the cystic duct extends to the left, somewhat ventrally and anteriorly (fig. 16). The gall-bladder is pear shaped (fig. 13) with its large, blind end projecting slightly dorsally and to the right but mainly caudalward.

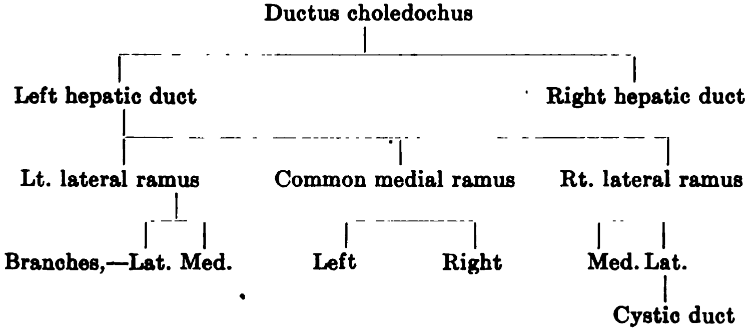
f. Summary of the development of the biliary apparatus. In summarising the development of the hepatic ducts a table of the ducts as found in the various models will bring out more clearly their relations to the main duct. Table 3a to 3d shows the principal variations found in the hepatic and cystic ducts.

TABLE 3a



Or, in case of anastomoses of the medial rami, as was found in two embryos of 14 and 20 mm. length and two older Amblystoma of 7 and 10 cm. length respectively, the following table is given:

TABLE 3b



The cystic duct is attached as here shown:

TABLE 3c

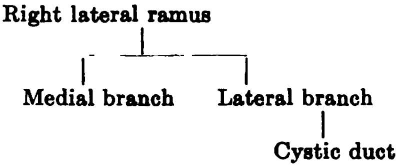
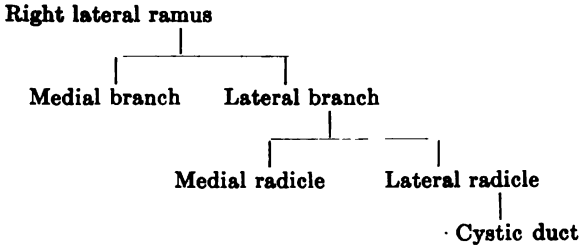


TABLE 3d



or as found in a 35 mm. embryo and one of the larger Amblystoma.

From these tables it will be seen that sometimes the right and left medial rami are joined. The division of the common medial ramus is into right and left branches. In their position and final division these branches are the same as the right medial and left medial rami. As will be seen in figures of the different models, the smaller embryos did not have all of the divisions and subdivisions marked in the tables. In figure 39, for instance, the right branch of the common medial ramus shows no further division, the left branch only one. Further division of both is seen in the 20 mm, stage (fig. 43). The division here, however, is more into dorsal and ventral radicles, due to the more marked lateralward shifting of the liver and the ducts. The extreme of this lateral shifting is seen in figure 44, where the left hepatic duct is almost ventral to the right. The left lateral ramus in a 45 mm. embryo does not hold such a ventral position with reference to the left medial.

There seems to be no definite rule in regard to the anastomosing of ducts. In a 35 mm. embryo they are the most frequent and here apparently because the ducts were crowded so close together. That the right and left medial rami sometimes join and form one duct is seen in the models of a 14 and a 20 mm. embryo, also in the graphic reconstruction of a 7 cm. and 10 cm. Amblystoma. It would seem this fusion of the ducts is quite probably due to crowding.

The definite position of the hepatic ducts with reference to the portal vein is seen for all embryos (figs. 8 to 12). The same relation is also found in the adult. As a rule there is a branching of the hepatic ducts corresponding to the division of this vessel. In the developing embryo the ducts are found usually to the right of and ventral to the portal vein.

From the usual description of the biliary apparatus in the frog it would seem that there is a fairly close correlation in the main features between these two amphibians. The figures of Ecker, Wiedersheim and others show a gall-bladder connected to a right hepatic duct. There is also a left hepatic duct, the two uniting in the pancreas and forming a ductus choledochus which, as usually described, is joined by the pancreatic duct. In no case in Amblystoma were two cystic ducts found as is shown for the frog. The division into rami in the frog as far as the ducts have been figured, seems to be somewhat different from that found in Amblystoma. The more marked divisions of the liver into several lobes may partially explain this. The duct-system as found in Necturus is quite different. Kingsbury here described three hepatic ducts opening into the gut. These anastomosed with each other and two were joined by the ventral pancreatic ducts. The third is a duct direct from the gall-bladder which, however, anastomoses with the other hepatic ducts. Grönberg ('94) described three hepatic ducts which unite with the cystic duct and form a ductus choledochus in Pipa americana.

Bates ('04) has described the hepatic ducts in Amblystoma. According to his description there are four heaptic ducts, two of which join the bile-duct in its course through the pancreas and the other two just as it opens into the intestine. It is possible that the two he found joining the bile-ducts are the right medial and lateral rami, and the other two, the left medial and lateral rami. In that case the ductus choledochus and the right and left hepatic ducts were very short as was found in some of the material used in this work. Or it may be that the two ducts which joined the bile-duct as it opened into the intestine are the two pancreatic ducts which have not fused until just at the ostium of the hepatic duct. The first two ducts then would be the right and the left hepatic ducts. I have never seen the cystic duct (bile-duct as Bates terms it) open directly into the common hepatic duct.

From the models and drawings it will be seen that the gall-bladder at first has a wide dorsal communication just caudal to the hepatic lumen. As this communication constricts there is formed a short large cystic duct extending dorsally into the right hepatic duct. With further growth and division the cystic duct extends more and more to the left until at the 15 mm. stage it is almost horizontal and at the 20 mm. stage projecting ventrally and somewhat anteriorly. Its earliest attachment is to the ventral surface of the common bile-duct, but in the lateralward shifting of the whole liver its attachment goes to the left side of a right hepatic radicle. The connection of the cystic duct to the gall-bladder in early stages is to its dorsal surface about midway between cranial and caudal pole. Somewhat later the connection is nearer the cranial end and usually reaches the extreme anterior end. The cranio-caudal growth of the gall-bladder has kept pace with the lengthening and differentiation of ducts in the 13 to 14 mm. stage. From the relations in a 15 mm. embryo it appears that the gall-bladder has shifted anteriorly. In this case the hepatic ducts have lengthened more than the gall-bladder. At 20 mm., however, there has been a marked increase in cranio-caudal growth of the gall-bladder so that it is almost as long as the ducts.

Beginning about at this stage the cystic duct is again attached nearer the anterior end of the gall-bladder. This may be taken as evidence that the cystic duct really shifts in its attachment to the gall-bladder. This seems to be borne out in some cases by the fact that its attachment to the hepatic ducts is to a division of the lateral branch of the right lateral ramus instead of to the lateral branch proper. In some cases where the lateral branch is quite long the attachment may have remained to it.

Whether the gall-bladder originates from the early hepatic anlage or from the gut has caused much discussion. As said before Piper ('02) thought this a matter of interpretation. The more marked furrow caudal to the gall-bladder might be taken as evidence of its belonging to the hepatic anlage, also the fact that the same type of yolk-laden cells form hepatic tissue and gall-bladder. That it, at least is directly caudal to the hepatic anlage is proven by the early connection of its duct to the common bile-duct.

The connection of the cystic duct probably depends to some extent on the extent of growth and division of the hepatic ducts. It will be remembered that in the earlier stages the cystic duct opens into the common duct, then into the early right hepatic. In the further growth and division of the right hepatic duct the cystic duct becomes attached to one of its radicles. As noted above, the cystic duct opens into the lateral branch of the right lateral ramus in all of the embryos studied except one, which was 35 mm. long.

That there is considerable variation in the relative dorsoventral position of these main hepatic ducts is to be expected.

However, in general, a study of the models shows a close similarity in their positions. There is a constant rotation of the liver towards the right and with this is a similar one of the hepatic ducts. In this rotation the right ducts come to be more dorsal in position, the left more ventral. The right lateral divisions would thus be dorsal to the right medial and the reverse should be true for the left. In general such an arrangement is found. A variation in the length of the different ducts is present. However, there is qute a definite relation in the total lengths of ducts in the different embryos. In a 15 mm. embryo the common duct is quite short but the greater length of the hepatic ducts compensates for this reduction. In a 35 mm. embryo the common duct is long, the hepatic ducts and their radicles divide shortly.

III. THE DEVELOPMENT OF THE PANCREAS AND PANCREATIC DUCTS

1. *Literature*

The literature concerning the development of the amphibian pancreas like that regarding the liver is divisible into two periods, and Goette's work ('75) may again be said to mark the beginning of the newer one. The older observers mainly considered the pancreas as a part of the liver, or a modified lobe of that organ.

A list of the investigators describing the development of the pancreas will be found included in the tabular classification of the literature on the development of the liver (table 2).

Goette ('75) in his studies on the development of the Bombinator recognized three distinct pancreatic anlagen, two ventral and one dorsal. The dorsal one he described as placed just caudal to the gastroduodenal loop. The two symmetrical ventral anlagen develop from the primitive hepatic duct. Of these the right grows dorsalward to join the ventral growing dorsal anlage. The right duct changes in position until it opens into the left side of the hepatic duct. The united right and left duct then separates from the common bile-duct. Apparently

Goette considered the left outpouching as a rudimentary one. Later the dorsal duct disappears, thus leaving but one permanent pancreatic duct.

Balfour ('81) and Hertwig ('88) described a dorsal outpouching of the gut wall caudal to the level of the common bile-duct.

The development of the pancreas in both Urodela and Anura was described by Goeppert ('91). In both he found as Goette had described, one dorsal and two symmetrical ventral outpouchings. A constriction of the early dorsal outgrowth forms a duct, while folds and ridges developing on the blind end give rise to the glandular tissue. The right and left ventral ducts unite on the right side of the common bile-duct. However, he found two pancreatic ducts opening into the common bile-duct in an adult, also three pancreatic ducts that fused immediately before opening into the common bile-duct. The numerous dorsal ducts which he found in the adult urodeles he explained are of secondary origin. He, too, found only one pancreatic duct persisting in adult Anura.

Marshall ('93) stated that in the frog the pancreas developed as a pair of hollow outgrowths caudal to the liver. Later the ducts shift and open into the bile-duct instead of, as at first, into the intestine.

Minot ('93) in his text book of embryology, mentioned that in urodeles the dorsal duct persists, and in Anura only the ventral duct.

Weysse ('95) and Stöhr ('95) both agreed with the description of Goette and Goeppert. Stöhr was especially interested in the dorsal pancreatic anlage. He did not find a double dorsal pancreas as had v. Kupffer ('92) in one of the ganoids. He believed that the caudal dorsal pancreatic anlage described by v. Kupffer is part of the hindgut. Brachet ('96) reviewed the descriptions of earlier investigators.

Woit ('97), a student of v. Kupffer, and probably influenced by his views, in his work on the development of the spleen stated that the dorsal pancreas gives rise to the spleen as well as to a part of the adult pancreas in urodeles. He described two persisting ducts in urodeles.

Gianelli ('99) described an "intrahepatic pancreas" in Triton, the tubules of which are in intimate relation with the liver-tubules, and it is stated by him are continuous with them.

Reuter ('00) made mention also of the early appearance of the dorsal and ventral pancreas. Both arise from the anterior part of the midgut region (Anfangsdarm) from the yolk-cells. The pancreas, as well as the liver, is found in the gastro-duodenal loop as soon as this is formed, and both are at first to the right and dorsal to the intestinal spiral.

Choronshitzky ('00) described in Necturus and the frog two lateral outpouchings from the early hepatic duct. These two lateral outpouchings form the ventral ducts, later they unite posterior (ventral) to the common duct. He described two ducts in the adult urodeles.

Gianelli ('02) described three distinct pancreatic anlagen in Triton. The dorsal anlage develops first. The ventral anlagen develop from the posterior end of the hepatic outpouching as two masses of vitelline cells into which the lumen of the hepatic evagination later extends. The right and left outpouchings both fuse with the dorsal pancreas. The ventral pancreatic duct formed from both pancreatic anlagen opens into the hepatic duct. The left pancreas remains in intimate relation with the liver.

Braun ('06) described the early development of the pancreas in Alytes obstetricans. He found a dorsal and two ventral pancreatic anlagen, which are first to be recognized as swellings of the yolk-gut wall and by the more numerous nuclei. The ventral anlagen are caudal to the anlage of the hepatic duct. The right ventral pancreas is somewhat more caudal than the left, joins the dorsal pancreatic outpouching and later joins with the left ventral pancreas. The dorsal outpouching looses its connection with the yolk-gut soon after coming in contact with the right ventral pancreas. The cells forming the pancreas are at this time still undifferentiated yolk-cells. Differentiation of the cells and glandular development take place at the same time. The early ventral outpouchings develop into the pancreatic ducts which unite just before opening into the gut to the right of the hepatic duct. The pancreas in the adult lies in the gastroduodenal loop.

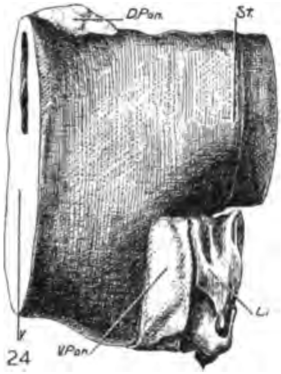
Eycleshymer and Wilson ('10) described the two ventral anlages as dorso-lateral to the ductus choledochus. These appear some time after the single dorsal anlage, and union with the dorsal pancreas does not take place until the embryo reaches a length of about 29 mm. They found that the dorsal duct opens into the duodenum just caudal to the stomach, and also mentioned two ventral ducts.

It is generally agreed by those who have described the duct system in the adult urodeles that at least two ducts persist. Hyrtl ('65) by means of injection in adult Cryptobranchus found two pancreatic ducts, one of which joined the hepatic duct. Oppel ('89) in Proteus has described an anterior and a posterior set of ducts, the latter emptying into the ductus choledochus. Kingsbury ('94) found one anterior duct opening just behind the pylorus, and two caudal ducts which open separately into the ductus choledochus. One pancreatic duct has been described as persisting in adult Anura. Bates ('04) stated that the pancreatic ducts join the hepatic ducts as they pass through the pancreas.

2. *Early development of the pancreas and pancreatic ducts*

As stated in the description of the liver, the pancreas develops later than that organ. A well marked dorsal pancreas is to be found in embryos 8 mm. long. A mass of cells in the dorsal wall of the enteron is separated by a distinct transverse furrow from the anlage of the stomach in front and from the yolk-mass behind. Mitotic figures are to be found in this mass of cells.

In embryos about 9 mm. long there are three pancreatic anlagen, two ventral and one dorsal, as has been described for other amphibia. The two ventral anlagen appear as evaginations posterior to the hepatic outpouching and caudal to the ventral-lying gall-bladder. The evaginations of the pancreas on the ventral wall of the gut extend in a longitudinal direction for some distance. Anteriorly there is quite a distinct furrow between the liver and the pancreas. A model of this stage (fig. 24) shows the outpouchings of the pancreas and of the gall-bladder and liver anteriorly. Posteriorly there is no sharp demarcation of the pancreas from the yolk-mass and gut. A model of the lumina of the pancreatic evaginations and of the gall-bladder and hepatic ducts makes the position of the different parts with reference to the antero-posterior plane more clear (fig. 25). At this stage the pancreatic anlagen are caudal to the gall-bladder which is directly anterior to the right pancreatic evagination.



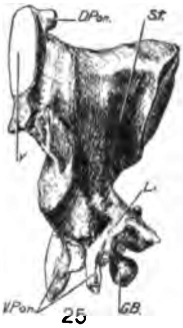
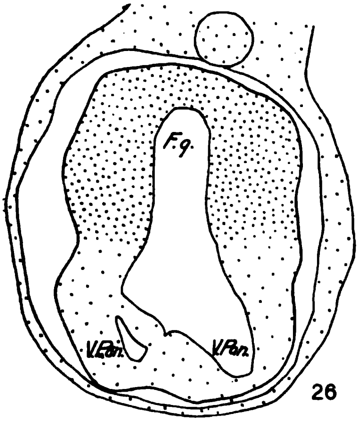


Fig. 24 Lateral view of a reconstruction of the pancreatic anlagen of a 9 mm. embryo. × 40. *D.pan.*, dorsal pancreas; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk-gut.

Fig. 25 Lateral view of a reconstruction of the lumina of the gut and pancreatic anlagen. × 40. *G.b.*, gall-bladder; other abbreviations, as in figure 24.

The pancreatic evaginations extend farther ventrally than do the hepatic ducts and the gall-bladder.

The right and left pancreatic anlagen are separated anteriorly by a slight ventral furrow. Caudally the two evaginations are apparently fused as the area between them is bulged ventrally. The evidence of a division into two evaginations is much more clear in a view of the model of the lumina of the pancreatic anlagen (fig. 25). This is also brought out clearly by the figure of a section taken about 80μ caudal to the gall-bladder (fig. 26). In this figure one sees the two very definite evaginations, and that they are separated as far as the transverse width of the gut will permit. On the lateral side there is an indefinite furrow at about the level of the dorsal margin of the liver extending caudalward in the wall of the gut and yolk-mass. This marks the upper limit of the ventral pancreatic anlagen (fig. 24). In



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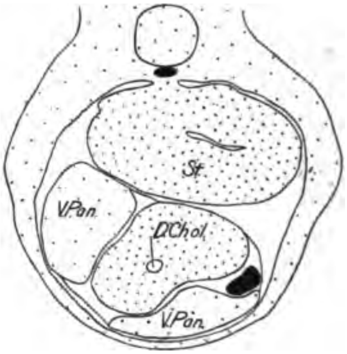

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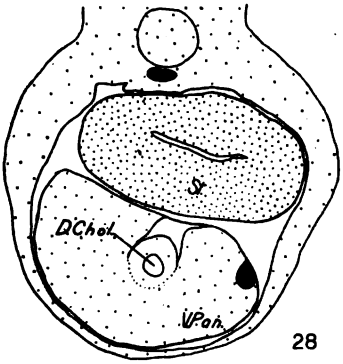

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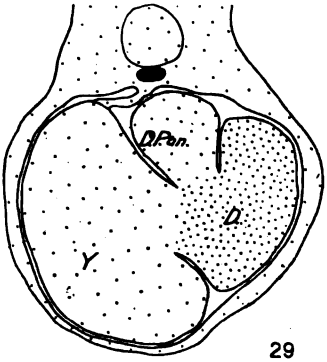

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Fig. 26 Drawing of a section through the ventral anlagen of an embryo 9 mm. long. × 40. *F.g.*, foregut; *V.Pan.*, right and left ventral pancreases.

Fig. 27 Drawing of a section through the ductus choledochus of an 11 mm. embryo. × 40. *D.chol.*, ductus choledochus; *St.*, stomach; *V.Pan.*, ventral pancreas.

Fig. 28 Drawing of a section about 80μ anterior to the preceding. × 40. For abbreviations, see figure 27.

Fig. 29 Drawing of a section through the dorsal pancreas of an 11 mm. embryo. × 40. *D.*, duodenum; *D. pan.*,dorsal pancreas; *Y*, yolk gut.

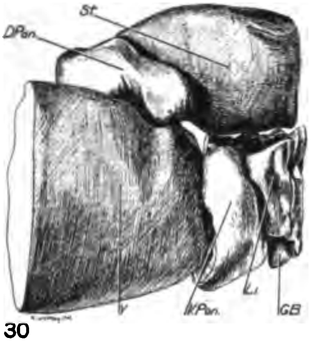
figure 26 the lumina of the two ventro-lateral pancreatic diverticula open widely into a common lumen which connects dorsally with the gut-cavity. Anteriorly at about the caudal end of the gall-bladder this lower common lumen is separated from the lumen of the intestinal anlage as shown by the model of the lumina of the ducts (fig. 25) as well as by the figure of a model of the hepatic ducts (fig. 20). In the section figured (fig. 26) the lower part of the right evagination is separated from the main lumen by cells. The next section anteriorly shows the left lumen also cut off. The pancreatic lumina thus very early extend somewhat forward.

The dorsal pancreatic anlage, as shown by both models (figs. 24 and 25), is median and further caudalward than the ventral. As seen in figure 24 it seems to be an elevated portion of the wall of yolk gut. The anterior and posterior furrows separating the anlange from the stomach and gut are not so prominent in this specimen. A model of the lumen shows it to be directed forward (fig. 25). Stöhr's statement that there are no evidences of double dorsal pancreatic anlagen in any stages as has been described in the ganoids by v. Kupffer is true also for Amblystoma. The dorsal anlage at this time is short in its craniocaudal diameter. It is, however, further developed than the ventral anlagen. Its ventral margin is limited by a slight groove at the anterior end. Caudally this groove is not present. Figure 24 as well as figure 25 shows that there has been quite an increase in the dorso-ventral diameter of the intestine. That the ventral part is becoming constricted from the dorsal is shown by both models and was pointed out in the description of the development of the liver. From the anterior end of the ventral part of the gut is the hepatic outpouching, caudal to this and on the right side the gall-bladder, and still farther posteriorly the ventral pancreatic anlagen. Caudal to these evaginations again the gut lumen takes a more dorsal position.

In 10 mm. embryos the dorsal pancreas is much more prominent. The furrow separating it from the stomach is quite deep. Also the caudal furrow is well marked. Mitotic figures are more numerous than before. The cells lining the evagination are columnar in type but still contain considerable yolk. The lumen extends a very short distance forward.

In an 11 mm. embryo there has been considerable further development of the midgut region. The stomach has differentiated to some extent. It has flattened dorso-ventrally, and its posterior end is constricted and shifted to the left. The duodenum extends ventrally to the left and has an anteriorly directed portion which forms, with the stomach, the gastro-duodenal loop. At its anterior end which Brachet ('95) has termed the 'seconde courbure' in Axolotl the duodenum turns to the right and is continuous with the caudal-extending yolk-mass. Here at the cranial end is the ventral pancreas. The pancreatic area appears at this stage as a narrow zone of the gut marked off by furrows, anteriorly from the hepatic area and posteriorly from the duodenum and yolk (fig. 30). The pancreatic ducts are short and extend ventro-laterally from either side of the common duct. These ducts are caudal to that part of the common bile-duct which gives off towards the right ventral side the cystic duct and anterior and somewhat dorsally two lateral hepatic ducts. The groove separating the anterior end of the pancreas from the hepatic tissue is well marked. The gall-bladder extends downward and to the right of the midline between pancreas and liver. A drawing of a section near the anterior end of the duodenal loop shows what appears to be a constricted forward projection of the gut (fig. 27). Somewhat anteriorly the cells lining this constricted gut are of a tall columnar type heavily laden with yolk as are the cells lining the duodenum (fig. 28). This is the caudal end of the common duct. Posterior to the section figured one can see the two lateral parts of the pancreas distinctly separated from the constricted anterior end of the gut where the common duct is attached (fig. 27). About fifteen sections of 10μ anterior to this are the ventro-laterally projecting pancreatic ducts. The pancreas has grown both anterior and posterior to the ducts, but the greater growth has been forward (120μ caudalward and 140μ anterior). The left pancreas grows anteriorly sending a small projection to the left of the gall-bladder. A model of the pancreas of this stage shows it as a cap placed over the anterior end of the gut, distinctly separated from it by a groove and closely united to the liver which lies in front of it. The right side shows only slight indication of its later dorsal and caudal growth (fig. 30).

The dorsal pancreas forms an irregular elongated mass to the right of the gastro-duodenal loop, but extends somewhat caudal to it. . To the right of the pancreas and ventrally lies the large yolk-mass (fig. 30). The dorsal pancreas is now relatively and actually nearer the ventral pancreas than in earlier stages. There is as yet little evidence of any ventral growth of the anterior end. The duct as in the younger stages lies mainly in the cranial part of the mass forming the dorsal pancreas. It extends to the right and dorsalward and is nothing more than a constricted part of the evagination. As seen in figure 29, it is attached to the archenteron near the large yolk-gut. The segment of the gut to which the dorsal pancreatic duct is attached is the caudal end of the duodenal loop in Brachet's ('95) 'ascending limb' which posteriorly is completely constricted from the ventral yolk-mass. Its attachment here, then as is shown by later stages, is to the dorsal wall of the duodenum.



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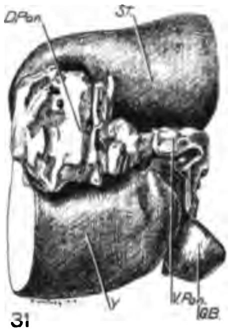

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Fig. 30 Lateral view of a model of the pancreatic anlagen of an 11 mm. embryo. × 40. *D.pan.*, dorsal pancreas; *G.b.*, gall-bladder; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk gut.

Fig. 31 Lateral view of a model of the pancreas of a 13 mm. embryo. × 30. *D.pan.*, dorsal pancreas; *G.B.*, gall-bladder; *St.*, stomach; *V.pan.*, ventral pancreas; *Y*, yolk-gut.

The development of the duodenum and the gastro-duodenal loop has been described by Goette and others who described the changes which bring the opening of the dorsal pancreatic duct nearer to the pylorus than the ventral ducts. Goeppert ('91, p. 113) stated concerning this: "—so erhält mann in den Schnitten die dorsale Anlage später als die ventralen Anlagen. Wenn mann aber die schräg absteigende Richtung des vorderen Schenkels der Gastroduodenalschlinge berücksichtigt, sieht mann leicht dass das dorsale Pankreas trotzdem einem noch etwas vor der Mündung des Leberstieles gelegenen Theil der Darmwand angehört."

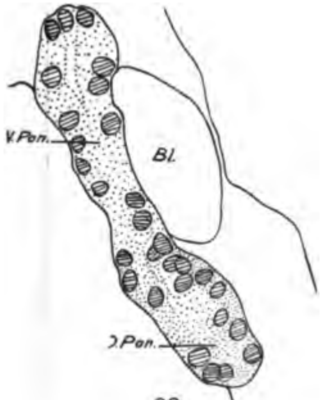
Brachet ('95) has described the position of the digestive tract in young axolotl. The duodenum continuing caudally from the stomach he has termed the first descending limb; the caudal turn, the 'premiere courbure;' then ascending limb, 'seconde courbure,' and second descending limb.

The dorsal and ventral pancreatic anlagen at this stage are composed of masses of cells still containing many yolk-granules. The ducts of both appear as constricted portions of the outpouching connecting them with the duodenum and the common bile-duct.

During the 12 and 13 mm. stages the dorsal pancreas comes in contact with the ventral one. The dorsal pancreas forms an irregular mass lying to the right of the duodenum and dorsal to the caudal yolk-mass. A small part extends anteriorly and somewhat ventrally and comes in contact with the right pancreas. In a 13 mm. embryo the two masses are fused (fig. 31). The acini of the two actually fuse as is shown by figure 32. The ventral pancreas is the smaller. A small part of the left ventral pancreas lies along the left side of the gall-bladder and anterior to the duodenum (fig. 45). The hepatic ducts extend through the ventral pancreas anteriorly to the liver but have no connection with the former. The right ventral pancreas in figure 31 has not grown dorsalward to any extent to join the dorsal pancreas. The dorsal pancreas in this case has grown ventrally and to the right to join the ventral pancreas.

The dorsal duct is now well developed. It extends dorsally and slightly to the right from the right dorsal side of the duodenum near its caudal turn. The duct divides shortly sending out branches in all directions.

The ventral ducts in a model of a 13 mm. embryo come off laterally from the hepatic ducts and immediately divide into smaller rami. As a rule the ventral ducts at this stage fuse into one tube ventral to the common duct. The ducts may join the ventral wall of the common duct or the anterior end of the gut, where the common bile-duct opens into the duodenum (fig. 45).



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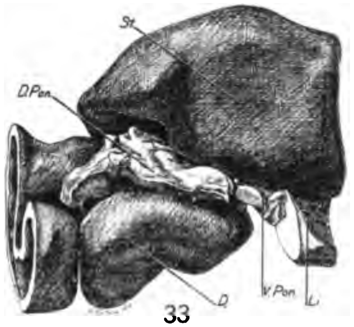

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Fig. 32 Drawing of a section showing the united acini of the dorsal and right ventral pancreas. × 180. *D.pan.*, from dorsal pancreas; *V.pan.*, from ventral pancreas; *Bl.*, blood vessel.

Fig. 33 Lateral view of a model of the pancreas of a 15 mm. embryo. × 30. *D.pan.*, dorsal pancreas; *D.*, duodenum; *Li.*, liver; *St.*, stomach; *V.pan.*, ventral pancreas.

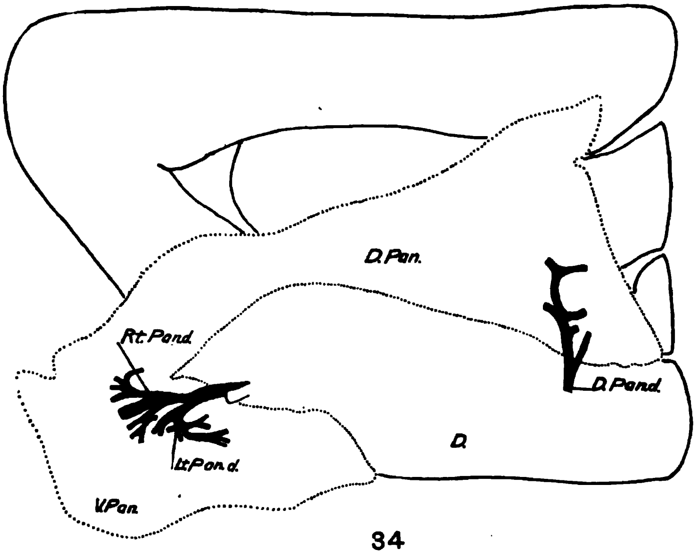
In a 15 mm. embryo as well as in later stages the dorsal pancreas forms the larger part of the whole organ. It joins the right ventral pancreas by a neck of tissue, which is larger than in the preceding stages (fig. 33). The ventral mass is crescentic in transsection and lies just below the stomach, and anterior and somewhat dorsal to the anterior end of the duodenum (fig. 45). A part of the ventral pancreas extends anteriorly along the left side of the gall-bladder. The pancreatic duct opens into the gut ventrally and slightly to the left of the common bile-duct (figs.

42 and 46). The pancreatic duct directly divides into two branches, a right and left, which end shortly. The dorsal duct has the same position as in the preceding stage.

In the following stages there is an increase in the size of the whole pancreas. The dorsal portion comes to lie more and more dorsal to the duodenum and along the right wall of the stomach, while the ventral portion increases in size anteriorly, in front of the anterior duodenal loop.

A description of the parts in a 35 mm. embryo is given as typical of the further development of the pancreas. In this stage the anterior part of the pancreas lies ventral to the gall-bladder and is embedded in the peripheral part of the liver (fig. 7, *B*). This part later probably forms the intrahepatic portion of the pancreas described by Gianelli ('99). Somewhat caudally it is considerably larger in section and separates the liver into dorsal and ventral portions (figs. 11 and 12). In this region just caudal to the gall-bladder the hepatic and ventral pancreatic ducts lie embedded in the pancreas. The pancreas is rather prismatic in cross section, its medial side lying along the right side of the stomach, its lateral dorsal side bounded by the duodenum. Slightly anterior to the ostium of the common duct the pancreas is divided into two masses, one lying ventral and somewhat to the left of the duodenum, the other to the left of the duodenum between it and the stomach (fig. 7, *C*). This latter mass joins the anteriorly directed portion of the dorsal pancreas. The remainder of the ventral pancreas now lies to the lower right side of the stomach, with a part projecting caudalward along the ventral wall of the duodenum (fig. 34). The dorsal pancreas is caudal to the ventral and takes a more dorsal position, until it comes to lie above the duodenum which has migrated downward. In cross section the dorsal pancreas extends from the lower right side of the stomach almost to its dorsal margin. About half a centimeter from its caudal end the pancreas forms a very small mass, triangular in section, dorsal to the duodenum.

The ducts of the ventral pancreas of a 35 mm. embryo are shown in a graphic reconstruction in figure 34. The ventral



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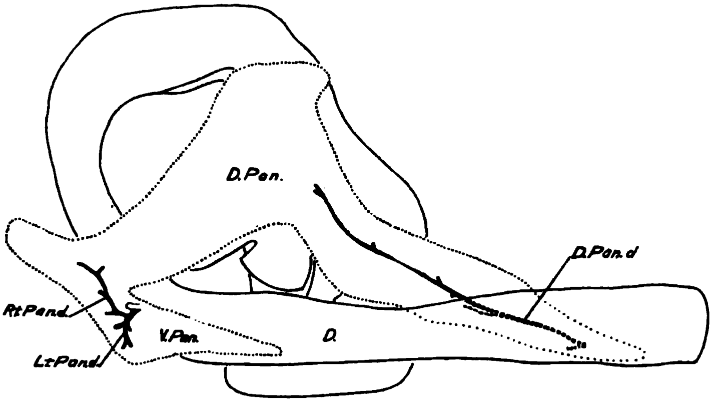

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Fig. 34 Graphic reconstruction of the pancreas and pancreatic ducts of a 35 mm. embryo. × 20. *D.*, duodenum; *D.pan.d.*, dorsal pancreatic duct; *D.pan.*, dorsal pancreas; *Lt.pan.d.*, left ventral pancreatic duct; *Rt.pan.d.*, right ventral pancreatic duct; *V.pan.*, ventral pancreas.

Fig. 35 Graphic reconstruction of the pancreas and pancreatic ducts of a 20 cm. Amblystoma. × 5. For abbreviations, see figure 34.

duct arises with the common bile-duct from a fold in the lower left wall of the duodenum (fig. 7, *C*). It shortly separates from the common bile-duct and extends anteriorly along its right side. It is somewhat ventral to the hepatic duct and divides into dorsal and ventral ducts. The ventral duct shortly sends off branches caudalward to the left ventral portion and extends forward in the ventral anterior part (fig. 34). The dorsal of the two ducts divides several times into dorsal and ventral rami. Branches from the dorsal duct extend caudalward and to the right into the anteriorly directed portion of the dorsal pancreas. The dorsal pancreatic duct is given off from the upper left side of the duodenum near the caudal end of the pancreas and extends almost directly upward sending off several short branches anteriorly and posteriorly.

3. Description of the adult pancreas

In a 15 cm. Amblystoma the anterior end of the pancreas, which is somewhat triangular in section, is embedded in the liver to the right of the stomach. It lies along the upper concave border of the liver with the portal vein and has a small free surface lined with peritoneum. Caudal to this the pancreas lies to the left of the duodenum, between it and the stomach and enlarges in a dorso-ventral direction (fig. 35). Now it passes along the left wall of the duodenum but extends both dorsally and ventrally to it, the ventral portion being between the duodenum and stomach. About 5 mm. by sections from its anterior end, the pancreas divides into two masses, one along the left ventral surface of the duodenum and the other dorsal to it. The ventral mass remains in about the same position, and extends caudalward almost half the length of the body of the gland. At the caudal end of the ventral portion of the pancreas the dorsal mass which has shifted somewhat to the right and lies above other loops of the intestine as well as the duodenum, is divided into two or three irregularly-shaped lobes, one of which is dorsal to the duodenum and directly to the right of the stomach. This part of the pancreas extends a considerable distance caudalward. As the duodenum shifts downward and to the left with relation to the stomach, this portion of the pancreas lies more and more to the right of the duodenum and for a considerable distance it lies ventral to the stomach. The pancreas extends caudalward almost to the gastro-duodenal loop.

A short distance from the caudal end of the pancreas the dorsal pancreatic duct is connected to the right side of the duodenum (fig. 35). This duct extends forward almost to the point where the dorsal pancreas divides into several parts and gives off small branches as it comes to lie more and more in the dorsal part of the gland. The duct of the ventral pancreas joins the common bile-duct (fig. 35) where it opens into the left side of the duodenum or beside it. The pancreatic duct is ventral to the common bile-duct and almost immediately divides into right and left ducts. The left duct is the more ventral and soon divides into branches which turn ventral and caudally (fig. 35). The right duct extends anteriorly and upward and sends some branches into that part of the dorsal pancreas which joins the ventral.

4. *Discussion*

As has been found in other amphibia, the pancreas in Amblystoma is developed from three anlagen, two ventral and one median dorsal. As in other forms the dorsal develops earlier and, as Stöhr and others have stated, from only one evagination. This is found just caudal to the anlage of the stomach. In none of the embryos studied is there evidence of an outpouching toward the left as Goeppert ('91) described in another form. Greil ('05), however, in young Bombinator embryos reconstructed right and left dorsal pancreatic outpouchings. Older embryos show that there has been considerable growth of the dorsal pancreas in a cranio-caudal direction with the lengthening of the duodenum, and a division of the anterior end into several processes, one of which joins the ventral pancreas.

In the very earliest stages the ventral anlagen are caudal to the gall-bladder. Goeppert ('91) and Chronshitzky ('00) have described these anlagen as lateral to the cystic evagination in the forms studied by them. Greil ('05) reconstructed the pancreatic anlagen as well as the branchial pouches of 7 and 7.5 mm. Bombinator embryos and showed the ventral pancreatic evaginations as dorso-ventral to the attachment of the hepatic outpouching and described them as lateral to the hepatic anlage.

In Amblystoma the two ventral pancreases unite very early along their medial sides. Braun ('06) has described the union of the dorsal and the right ventral pancreas as occurring before the union of the right and the left ventral anlagen and Greil stated that the right ventral pancreas has united with the right dorsal in 7.5 mm. embryos. The appearance of considerable pancreatic tissue from the left side of the ventral anlage would indicate that there is growth from this evagination. Goette apparently thought this outpouching was rudimentary. The presence of a left duct in some 12–13 mm. embryos indicates that there is growth from the left side. Later stages show that there is more growth on the right side.

Although the lumen of the dorsal pancreatic anlage at first extends anteriorly, the duct later extends upward and in the adult again forward. Short lateral branches extend into the surrounding pancreas. The ventral ducts fuse to form a single one which divides into a right and left pancreatic duct. These again divide into smaller rami, of which some from the right side extend into the portion uniting with the dorsal pancreas. The dorsal and ventral ducts, however, always remain separate.

It is clear that in Amblystoma there is no complex pancreatic duct system as Oppel ('90) has observed in Proteus. In possessing a single dorsal duct Amblystoma resembles the Necturus as described by Kingsbury ('94). The posterior set of ducts emptying into the ductus choledochus as described by Oppel is quite different from the single ventral duct in Amblystoma. Nor does this conform to Kingsbury's description of two posterior pancreatic ducts each of which open into hepatic ducts. I cannot confirm Bates' ('04) observations concerning the several pancreatic ducts which he stated opened into the hepatic ducts within the pancreas. A table of the ducts as they have been described in various urodeles may be of interest.

As is well known the duct of the dorsal pancreas does not persist in Anura. However, Goeppert ('91) and Vogt and Yung ('94) have described several pancreatic ducts in the ventral pancreas some of which joined the ductus choledochus or hepatic duct. It is seen from the table that there is considerable variation in the pancreatic duct-system of the urodeles. The complex system of some forms may be due, as Goeppert suggested, to a later union of the lesser pancreatic ducts with the duodenum or common duct.

TABLE 4

Table of the pancreatic ducts in the various urodeles

Form	*Author*	*Dorsal pancreas*	*Ventral pancreas*
Cryptobranchus japonicus	Hyrtl ('65)	1 (?)	1, joining with ductus choledochus
Salamandra perspicillata Geotriton fuscus	Wiedersheim ('75)	1 (?)	1, joining the two hepatic ducts
Proteus anguineus	Oppel ('89)	10, 33	9, 11 forming network with ductus choledochus
Menobranchus lateralis	Goeppert ('91)		2, joining ductus choledochus
Salamandra maculata	Goeppert ('91)	1	1, joining ductus choledochus
Salamandra altra	Goeppert ('91)		3, joining ductus choledochus
Triton alpestris	Goeppert ('91)	2	1, joining ductus choledochus
Triton taeniatus	Goeppert ('91)		1, opening near ductus choledochus
Cryptobranchus japonicus	Goeppert ('91)	6	1, opening near ductus choledochus
Necturus maculatus	Kingsbury ('94)	1	2, joining separate hepatic ducts
Triton	Gianelli ('02)	1	1, joining hepaticocystic duct
Amblystoma	Bates ('04)	—	Several—joining various hepatic ducts
Amblystoma	Baumgartner ('15)	1	1, may or may not join ductus choledochus

The glandular portions of the ventral and dorsal pancreases fuse as is clearly shown in figure 33. This fusion of the glandular parts takes place immediately after the two parts come in contact. The union of the two parts is at first only a narrow neck of tissue, which remains small even in adults.

In a 35 mm. embryo and in smaller ones attention was called to a small part of the ventral pancreas lying below the gall-bladder and separating the liver into upper and lower parts.

The peripheral portion of the liver grows more rapidly and surrounds this part on the outer side. It then has a peritoneal surface only on the medial upper side. This corresponds to Gianelli's intrahepatic portion of the pancreas. However, as stated by Goeppert, the pancreatic and hepatic tissues are always clearly separate. Goeppert ('91) has given a description of the relations and lobes of the pancreas. The pancreas in Amblystoma resembles that in those forms which he described in having a ventral part or lobe caudal to the liver and in intimate relation with it, and a dorsal or caudal part. Kingsbury described five more or less distinct parts: The following table shows a correlation of the lobes of the pancreas which various investigators have described with those of Amblystoma.

TABLE 5

Table of the parts of the adult pancreas in Amphibia

Proteus......... Oppel ('89).....	Vordere Theil	Mittlere Theil	Hintere Theil		
Salamandra, Rana, etc....... Goeppert ('91)..	Dorsal oder vordere Theil	Ventral oder hintere Theil	Hinterste Theil		
Necturus....... Kingsbury ('94)	Lobe along intestine	Central part near gall bladder	Lobe along dorsal wall of liver	Lobe along splenic vein	Lobe along mesenteric vein
Triton.......... Gianelli ('02) ..	Corpo, estremità posteriore	Estremità craniale	Pancreas intraepatico		
Alytes obstetricanus......... Reuter ('06)....	Kopf	Wurzel			
Amblystoma...	Dorsal portion	Ventral portion	Portion along dorsal concave border of liver		

Goeppert mentioned that there were usually several other prolongations of pancreatic tissue. This is also true for Amblystoma, particularly from the anterior end of the dorsal portion were there several prolongations. It is to be remembered that 'vordere' is used by Oppel and Goeppert to express proximity to the oral end of the intestinal loop and not in the ordinary topographical sense.

IV. GENERAL SUMMARY

1. The liver begins as a median ventral projection of the lumen of the gut, then as an anterior outpouching from this lumen.

2. There is a later shifting of the posterior part of the liver to the right and dorsally, due to crowding of the stomach and development of the duodenum on the left.

3. A later growth on the left side results in an adult organ with right and left parts, the right side always remaining more dorsal on the lateral side of the stomach.

4. The ductus choledochus develops as the early anteriorly directed lumen from the gut.

5. The right and left hepatic ducts develop as divisions of the ductus choledochus and by division and growth form the hepatic rami and branches.

6. The gall-bladder begins as a median ventral outpouching of the posterior part of the liver-anlage. It is first widest laterally, then becomes larger in its cranio-caudal diameter, then its dorso-ventral and finally its longer axis is nearly transverse.

7. There is an early right lateral shifting of the gall-bladder as of the liver, due probably to the same causes. Along with this there is a constant shifting of direction of the cystic duct in keeping with the dorsalward migration of the gall-bladder.

8. The cystic duct is early closed off with the right hepatic and due to the caudalward growth and division of the hepatic duct is finally attached to the lateral branch of this duct.

9. The ventral pancreatic anlagen are ventro-lateral evaginations of the gut caudal to the cystic anlage. The dorsal pancreas—a single median dorsal evagination—forms the larger portion of the early pancreas, later it is a narrow lobe dorsal to the duodenum.

10. The ventral pancreatic ducts are constrictions of the two ventral pancreatic anlagen. Later these unite and form a single ventral pancreatic duct which opens into the common bile-duct or into the intestine at the side of the common bile-duct. The dorsal duct remains a single stem with short lateral branches.

11. There are two main parts or lobes in the adult Amblystoma pancreas, a dorsal and a ventral, with one or more lesser projections from these. An anterior extension of the ventral lobe is constant.

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PLATE 1

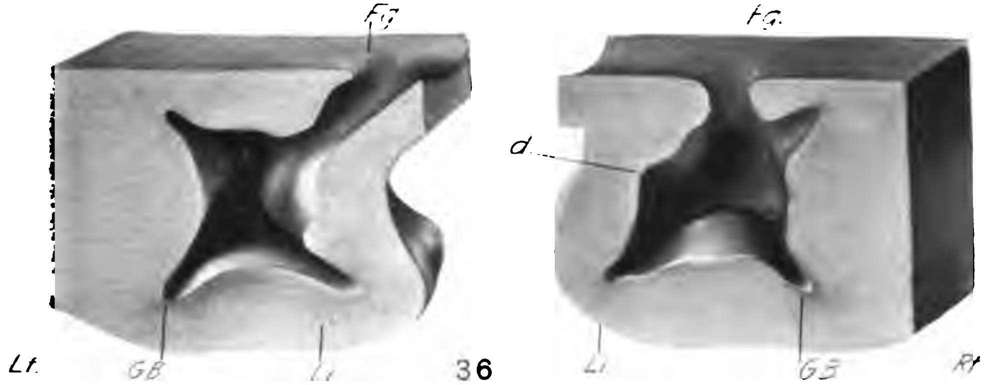
EXPLANATION OF FIGURES

36 Median view of right and left halves of a reconstruction of the liver of an Amblystoma embryo 7 mm. long. × 70.

37 Median view of right and left parts of a reconstruction of the liver of an embryo 9 mm. long. × 70.

38 Posterior view of a reconstruction of the liver of an embryo 9 mm. long. × 100.

d., early anlage of duct
D. chol., ductus choledochus
D.cy., cystic duct
D.m., dorso-medial duct
D.h.d., right hepatic duct
D.h.s., left hepatic duct
G.B., gall-bladder
Li., liver
Lt. Rt., left and right parts of hepatic anlage
v.l., ventro-medial duct



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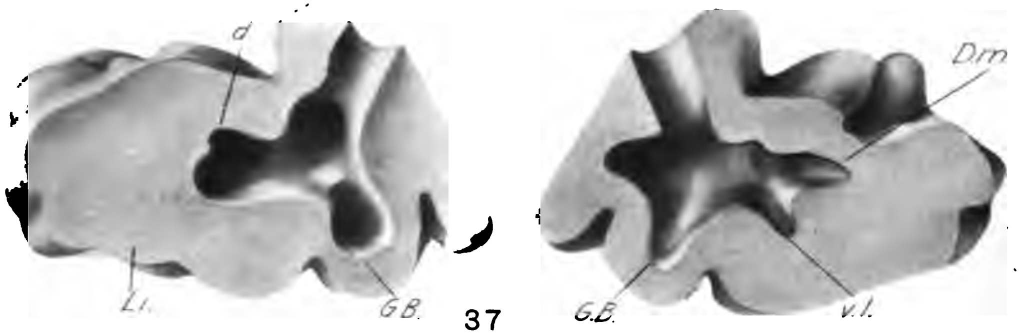

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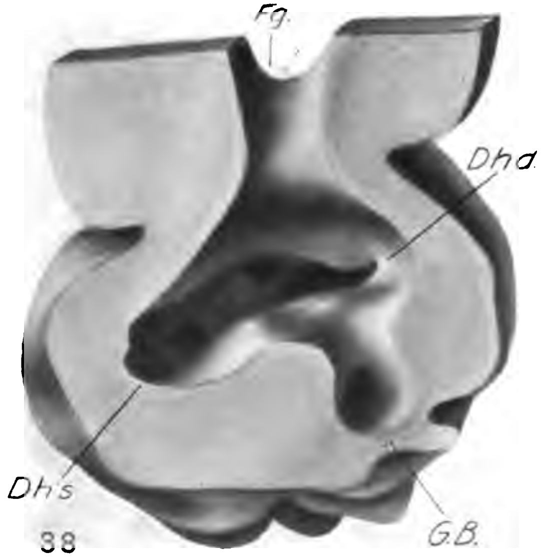

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PLATE 2

EXPLANATION OF FIGURES

39 Ventral view of a reconstruction of the hepatic ducts and gall-bladder of an Amblystoma embryo 14 mm. long. × 100.

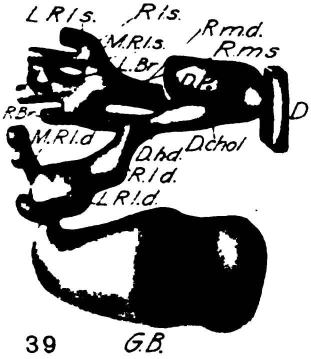
40 Dorsal view of the same reconstruction. × 100.

41 Ventral view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 13.5 mm. long. × 100.

42 Dorsal view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 15 mm. long. × 100.

43 Right ventral view of a reconstruction of the hepatic ducts and gall-bladder of an embryo 20 mm. long. × 100.

D., duodenum
D.chol., ductus choledochus
D.cy., cystic duct
D.h.d., right hepatic duct
D.h.s., left hepatic duct
D.P., pancreatic duct
g.b., gall-bladder
L.Br., left branch of common ramus
L.R.l.d., lateral branch right lateral ramus
L.R.l.s., lateral branch left lateral ramus
L.R.m.d., lateral branch right medial ramus
L.R.m.s., lateral branch left medial ramus
M.R.l.d., medial branch right lateral ramus
M.R.l.s., medial branch left lateral ramus
M.R.m.d., medial branch right medial ramus
M.R.m.s., medial branch left medial ramus
R.Br., right branch of common ramus
R.l.s., left lateral ramus
R.l.d., right lateral ramus
R.m.s., left medial ramus
R.m.d., right medial ramus
Z., extra duct in 13.5 mm. Amblystoma embryo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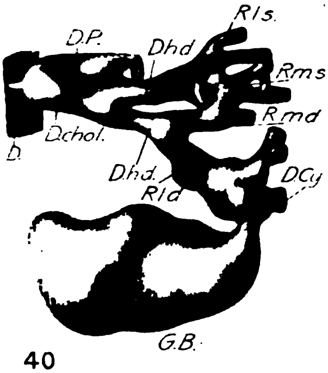
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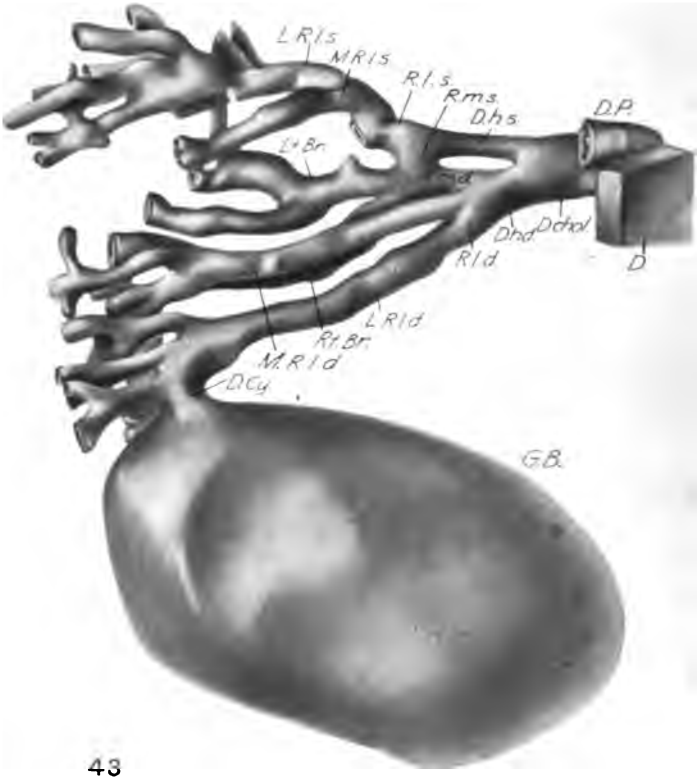
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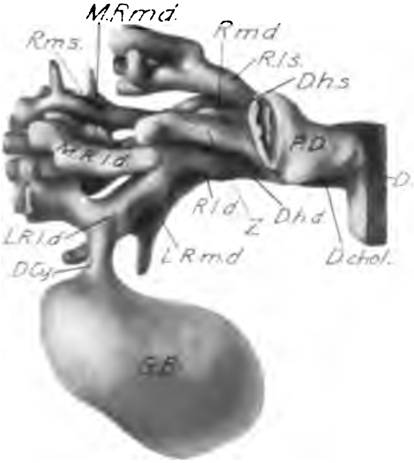
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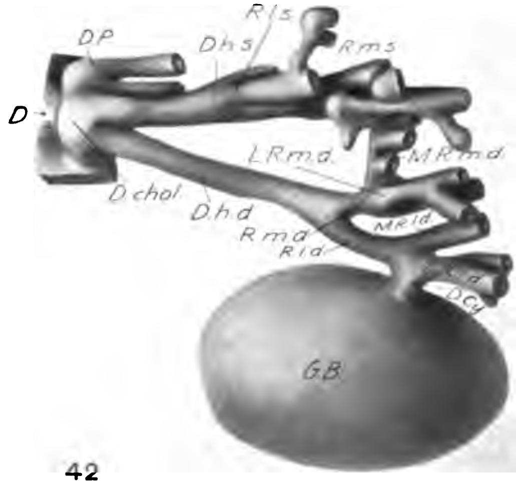
G.B.

41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R.m.d
R.l.d
M.R.l.d
L.R.l.d
D.Cy
G.B.

42

PLATE 3

EXPLANATION OF FIGURES

44 Right ventral view of a reconstruction of the hepatic ducts and gall-bladder of an Amblystoma embryo 35 mm. long. × 70.

D., duodenum
D.chol., ductus choledochus
D.cy., cystic duct
D.h.d., right hepatic duct
D.h.s., left hepatic duct
D.P., pancreatic duct
g.b., gall-bladder
L.Br., left branch of common ramus
L.R.l.d., lateral branch right lateral ramus
L.R.l.s., lateral branch left lateral ramus
L.R.m.d., lateral branch right medial ramus
L.R.m.s., lateral branch left medial ramus
M.R.l.d., medial branch right lateral ramus
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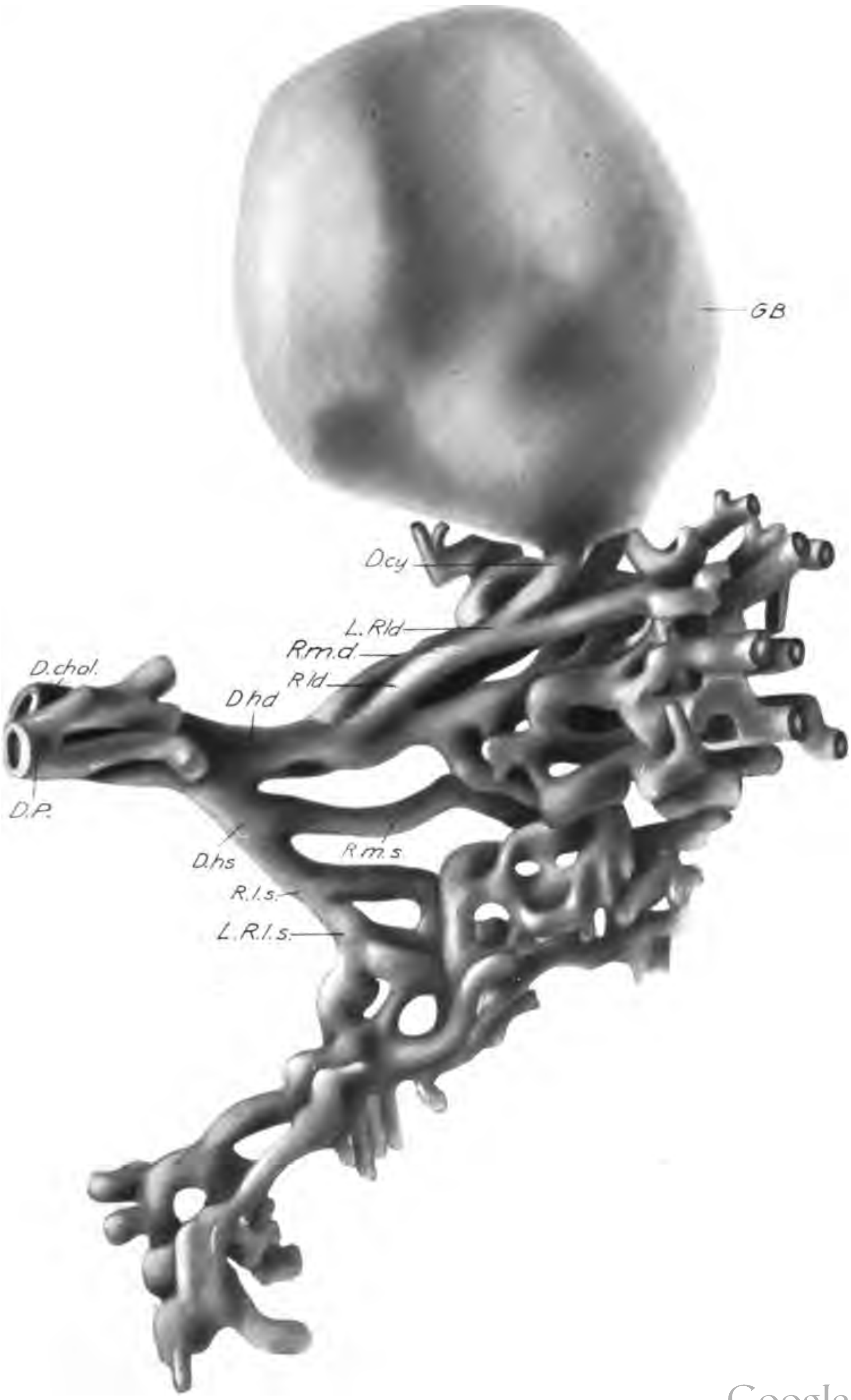
M.R.m.d., medial branch right medial ramus
M.R.m.s., medial branch left medial ramus
R.Br., right branch of common ramus
R.l.d., right lateral ramus
R.l.s., left lateral ramus
R.m.d., right medial ramus
R.m.s., left medial ramus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D.cy
L. Rld
Rm.d.
Rld
D.chol.
D.hd
D.P.
D.hs
Rm.s
R.l.s.
L.R.l.s.

PLATE 4

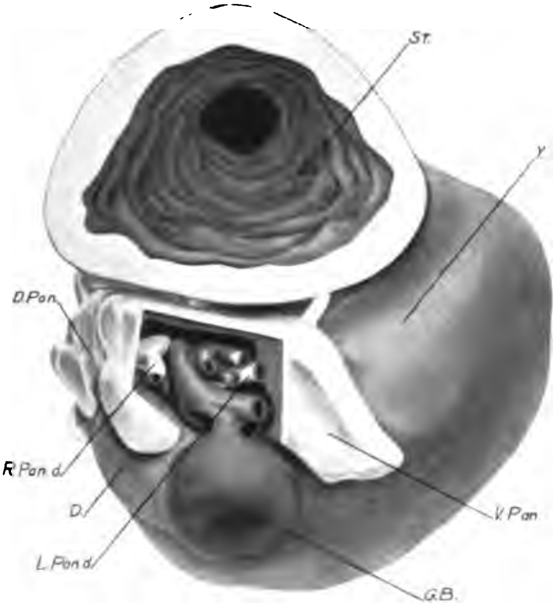
EXPLANATION OF FIGURES

45 Anterior view of the pancreas and ventral pancreatic ducts of a 13 mm. embryo. × 60.

46 Anterior view of the pancreas and ducts of a 15 mm. embryo. × 60.

D., duodenum
D.pan., dorsal pancreas
G.B., gall-bladder
Lt.pan.d., left ventral pancreatic duct
Rt.pan.d., right ventral pancreatic duct
St., stomach
V.pan., ventral pancreas
Y, yolk-gut
D.chol., ductus choledochus
V.pan.d., ventral pancreatic ducts.

For other abbreviations, see figure 45.



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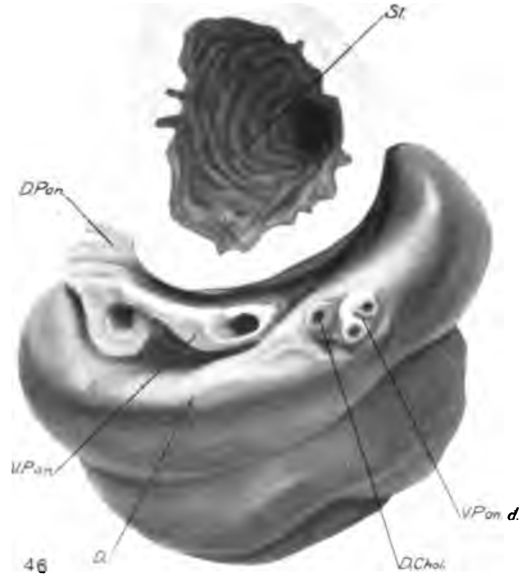

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THE GROWTH OF THE BODY AND ORGANS OF THE ALBINO RAT AS AFFECTED BY FEEDING VARIOUS DUCTLESS GLANDS (THYROID, THYMUS, HYPOPHYSIS, AND PINEAL).

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FOUR CHARTS

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I. INTRODUCTION

The present investigation was undertaken in the hope of throwing further light upon the relations of some of the ductless glands to the growth process in albino rats. During the experiment it became evident that the growth rate in the control rats was in many cases somewhat different from that which has been generally described as normal, so it became necessary to include incidentally the question of the normal growth rate.

The investigation was carried on in the Anatomical Institute of the University of Minnesota, under the direction of Professor C. M. Jackson, to whom my grateful thanks are due for his constant interest in the work and his many very helpful suggestions.

Since the ductless or endocrinous glands were first recognized anatomically, various methods have been applied in investigating their functional significance. The four most commonly used, are extirpation of the glands, their transplantation, injection of their extracts and the feeding of the glandular substances.

Feeding ductless glands in order to study the effects of the hyperactivity thus possibly produced has certain objections. The absorption is slower than when injections are made, and the danger of infection is lessened; but the substances fed may undergo digestive changes in the alimentary tract. That the active principles of the ductless glands are not necessarily destroyed by digestion, however, is proven by abundant experimental and clinical results (Gudernatsch '12, and Abderhalden '15 especially in thyroid feeding). The feeding method was selected for the present investigation.

II. LITERATURE

A general discussion of the literature of all of the ductless glands is given by Vincent ('12), "Internal Secretion and the Ductless Glands," and in the more extensive work of Biedl ('13), "Innere Sekretion." The hypophysis literature has been reviewed by Cushing ('12). "The Pituitary Body and its Disorders;" and the work on the thymus has been considered recently by Basch ('13), "Beiträge zur Physiologie und Pathologie der Thymus." A complete list of references to work done upon the albino rat is given by Donaldson '(15).

A preliminary report of the principal results of the present investigation has already been published (Hoskins '16).

Thyroid experiments

Iscovesco ('13) found that daily injections of thyroid extract stimulated growth slightly in young animals but decreased the weight of old animals. He found nearly 100 per cent hypertrophy (measured in grams per kilogram body weight) in various viscera. The extreme and uniform hypertrophy of the organs and especially that of the uterus is difficult to understand. The fact that the liver and the female kidney show no overgrowth is remarkable.

Magnus-Levy ('95) found that feeding thyroid may cause loss in weight in an animal.

Cunningham ('98) fed 'considerable amounts' of thyroid to various animals without noting any toxic effects.

Moussu ('99) reported that small doses of thyroid stimulate the rate of growth in young dogs, but that large doses are toxic.

Rudinger, Falta and Eppinger ('08) and Kostlivy ('10) found that feeding thyroid stimulates the suprarenal glands.

Bircher ('10 a) ('10 b) fed thyroid to young rats and found a retardation in growth and body weight, but an acceleration in the process of ossification.

Utterström ('10) reported an enlargement of the thymus in thyroid-fed rabbits.

Hoskins ('10 a) fed daily, for 15 days varying amounts (5–15 mg.) of thyroid to 18 young guinea pigs. Their suprarenal glands were 25 per cent heavier than those of 18 controls. The same author ('10 b) fed thyroid to pregnant guinea pigs. Many abortions and several still-born occurred. The newborn (apparently normal) young of the treated mothers weighed on the average 12 grams less than the controls. The hypophysis showed an average decrease in weight of 10 per cent, the suprarenals 26 per cent and 2 per cent for the females and males respectively; the ovaries 26 per cent; and the thyroid gland 18 per cent. The thymus was increased 58 per cent.

Carlson, Rooks and McKie ('12) and Ferrant ('13) fed thyroid to birds and mammals, including man. They concluded that large doses of thyroid are toxic. In the thyroid fed rabbits of Ferrant the heart, liver and pancreas showed degenerative changes.

Schäfer ('12) fed thyroid to young white rats, noting an increased food consumption, increased metabolism and acceleration of growth.

Gudernatsch ('12, '14) found that thyroid administered to a large number of tadpoles retarded growth (i.e., a toxic effect) but hastened metamorphosis of the limbs and tail. It is probable that this acceleration is due to an increase in the rate of the circulation and general metabolism. Lenhart ('15) reaches the same conclusion. The present writer, in a similar (unpublished) experiment (with larval frogs and Ambystomae) fed large doses and noted only the toxic effect. Coutronei ('14), West ('14), Morse ('15) Abderhalden ('15) and Romeis ('15) have recently confirmed in general the results of Gudernatsch.

Hewitt ('14) fed thyroid gland to white rats, noting a loss in weight.

Livingston ('14) found that feeding thyroid inhibits the hypertrophy of the hypophysis which follows thyroidectomy in male rabbits.

Gudernatsch ('15) fed thyroid to albino rats. This treatment retarded growth and interfered with pregnancy. The effect produced is probably due merely to the toxicity of thyroid, as Stockard ('13) obtained quite similar results in animals treated with alcohol.

Thymus feeding

For complete literature see Vincent ('12), Biedl ('13), Basch ('13) and Paton ('13).

Gudernatsch ('12) ('14) found that a thymus diet stimulated body growth in tadpoles, but retarded metamorphosis of the limbs and tail. Similar results in some cases were obtained by Romeis ('15) and Abderhalden ('15).

Gebele ('11) and Miss Hewer ('14) reported negative results with thymus feeding. In the latter's work, thymus, when fed (1 to 4 g. daily) to males, retarded the development of the testes in young rats and caused degeneration of the testes in adults.

Salkind ('15) reported stimulation of growth from feeding large amounts of thymus.

Hypophysis experiments

Cushing ('12) concludes that total loss of the anterior lobe of the hypophysis is followed by death and that partial loss is followed by obesity, sexual infantilism, and retardation of skeletal growth.

Frequent injections of hypophysis extract interfere with growth, (Cerletti '09, Fodera and Pittau '09). A loss in weight with no skeletal changes is reported by Franchini ('10) and by Crowe, Cushing and Homans ('10). A gain in weight after continued injections of hypophysis extract is reported by Delille ('09), and cardiac hypertrophy by Etienne and Parisot ('08); but Caselli ('00) obtained negative results.

Retardation in growth (especially skeletal) as a result of hypophysis feeding was reported by Thompson and Johnston ('05), Etienne and Parisot ('08), Sandri ('07), ('09) (posterior lobe), and Cushing and Goetsch (cited by Cushing ('12), Wulzen ('14) and by Pearl ('16) (anterior lobe) Abderhalden ('15), and Robertson ('16).

Negative results from feeding hypophysis are reported by Caselli ('00), Sandri ('09) (anterior lobe), Hoskins ('11), Aldrich ('12a) ('12b), Schäfer ('12), Lewis and Miller ('13), and Gudernatsch ('14).

Schäfer ('09) had thought that feeding small amounts of anterior lobe of the hypophysis favors growth. Goetsch ('16) from very few data reports that excessive dosage retards growth in young rats, whereas smaller dosage accelerates growth in body weight and especially the development of the sexual system; that this acceleration is due to the anterior lobe; and that feeding posterior lobe does not accelerate growth, and even retards sexual development. Robertson ('16a) reports that hypophysis extract accelerates growth after inanition.

So far as the literature shows, in none of the above experiments with hypophysis substance was a complete autopsy performed or complete histological examination made. Hallion and Alquier ('08) report hypertrophy of the suprarenals in rabbits, and no changes in the gonads. Wulzen ('14) noted in chickens fed fresh hypophysis that the long bones were shorter than in her control animals, and that the involution of the thymus was hastened by the treatment.

Pineal experiments

In experiments on growth, Dana and Berkeley ('13) reported that after injecting and feeding a few young animals with pineal substance, they noted growth acceleration. These results may be due to the pineal medication or merely to normal variation. Berkeley ('14) reported mental and physical improvement in backward children to whom pineal substance was administered.

Priore ('15) reports that young rabbits into which pineal extract was injected frequently, grew slightly more than the controls, but his results are probably within normal variability. In an older group the controls outgrew the experimental group. In none could any skeletal alterations be seen.

McCord ('14) fed pineal substance to a few chicks, young guinea pigs and pups. He reported an acceleration in their rate of growth, increased mentality in the dogs, and sexual precocity in some of the guinea pigs.

The same writer ('15) later reported that the growth of young guinea pigs which received daily 10 mgms. of pineal substance from adult cattle was delayed. Adult guinea pigs were not affected by calf pineal substance (dried), but the growth rate of young guinea pigs was accelerated by injections of extract of calf pineals. Pineal substance hastened sexual development in guinea pigs. Males were more susceptible than females to pineal treatment. The treated animals did not grow larger than normal adults and their growth was apparently proportional in all parts of the body. The 'sexual precocity' noted by McCord perhaps can be explained by the fact that in growing animals the sexual development normally tends to keep pace with somatic growth. In stunted animals sexual maturity is retarded as seen in inanition experiments (Jackson '15); conversely it is to be expected that a hastened somatic growth is accompanied by a corresponding development of the reproductive organs. This condition can hardly be called sexual precocity, especially if the sex organs are not relatively overdeveloped.

Dandy ('15) was able to remove the pineal body from young dogs with no apparent after-effects.

Many experimental data that have been published are worth very little to us because of incomplete records and also because animals of one strain kept under certain conditions have been used as control animals and checked against experimental animals of a possibly different strain kept perhaps under different conditions. It is also true that in many experiments, although all the animals are of the same strain and are kept under similar conditions, only a small number are used and these represent different litters. Since it has been shown by Jackson ('13) and King ('15) that in albino rats variability in body weight within a litter is only about half as great as general racial variability, the advantage of taking controls and experimental animals from the same litter is obvious. The apparent 'results' obtained in many experiments may very well be due merely to the abovementioned factors and not to the experiment itself.

III. MATERIAL AND METHODS

The albino rat (Mus norvegicus albinus) was selected for the experiment because it is a convenient form for use and more is known about the growth of this animal than of any other, owing to the work of Donaldson, Hatai, Jackson, Slonaker, Lowrey, King, and others. Owing perhaps to their diet and very favorable environment, most of the animals (especially the younger male groups) are somewhat larger than the average ordinary albino rats at corresponding ages. Twenty-nine litters were used but where litters contained less than four rats of a sex these individuals were usually rejected. A few rats were killed by the mother before weaning time. In all, 59 females and 73 males were fed and of these all of the females and 59 of the males were carefully autopsied at the termination of the experiment. While the number of observations is not large from the statistical point of view, and more would be necessary for final conclusions, it is believed that the present data are sufficient to establish certain points with a considerable degree of probability, and to furnish valuable evidence upon other points requiring further data. Most of the older animals which were born during the summer and early fall were smaller at the beginning of the experiment than were those born in the winter and spring. This is in accordance with previous observations. These initial differences generally persisted throughout the period of experiment (as likewise found by King ('15). Of the older litters, two of which were of purely local stock were quite large rats, whereas three litters which were of pure 'Wistar' stock were considerably smaller. Other litters used were of a mixture of these two strains.

The rats with few exceptions were weaned at 3 weeks of age, and kept in a well ventilated room in fairly large wire net cages, with wire net bottoms which allowed waste matter to drop through. The males and females were of course separated. All were fed (ad libitum) upon whole wheat (Graham) bread soaked in whole milk, a diet which seems to provide abundant nourishment, as shown by the rapid growth of the animals. During the first part of the investigation the rats were fed once a day and their water jars washed once a week. Later all of the animals were fed 3 times a day, the water jars washed daily and at all times the cages were kept clean. Each animal was given a mark of identification with picric acid and a separate growth record kept for it. In general, each rat was weighed at weaning time, and each day (before feeding) for about a week, but thereafter the interval between weighings was gradually increased.

The autopsy technique employed by Jackson ('13), with a few modifications, was used. The various organs were placed in a moist chamber when taken from the animal and weighed when all had been removed. The thyroid and thymus were freed from their capsules. In the younger groups of animals the mesentery and pancreas were removed from the stomach and intestines. These cases are marked (*c*) in the tables.

All the organs were weighed in closed containers to 0.1 mgm. The skeleton was prepared by heating the body (eviscerated and skinned) at 90 degrees centigrade for 2 or 3 hours in 2 per cent aqueous 'Gold Dust' (a proprietary soap powder) solution. The skeleton (including cartilages) was cleaned, drained carefully, and weighed, and then dried for 2 weeks (in an oven heated at 88 to 90 degrees centigrade) to constant weight and weighed again. This technique gives fairly constant results.

In the final averages shown in the various tables, extreme data are in a few cases excluded. (These extreme cases were probably due either to experimental error or to abnormal variation.)

The tables show only the averages of individual data. A copy of the original observations have been filed at The Wistar Institute of Anatomy and Biology, Philadelphia, where they may be consulted by those interested.

The material for feeding was obtained every 2 weeks by the author in person, from newly-killed calves 6 to 10 weeks of age, and ground fine in a kitchen meat-grinder. Some of the substance of each kind was spread out thin and dried before an electric fan at room temperature. The material was quite dry within 5 to 10 hours of the death of the calves. It was diluted with known amounts of milk sugar for measuring. A portion of each kind was kept fresh (at from zero to 5 degrees centigrade). No constant difference in the effects produced by the fresh and dry glands was noticeable, so in grouping the data the difference in the two conditions of the material administered was disregarded.

The rats used, were grouped to exclude so far as possible the error introduced by the racial variability. One rat of each litter (or one of each sex if both males and females of the litter were used) was kept for control, the remaining being distributed among the groups treated, in such manner that (so far as possible) each group contained individuals from every litter. There are five groups of each sex. To eliminate the 'age' factor, the data for each sex were subdivided into 'older' and 'younger' groups, as shown in the tables.

In calculating the results, two methods were used. In one the average of the percentage of the net body weight of each organ of each group of experimental animals was compared directly with the average value of that organ in the control group while in the second method a comparison was made with the Wistar reference tables as suggested by Donaldson ('15). In the latter comparison the gross body weight was used instead of the length because the weight of the organs of the controls corresponded somewhat more closely to Donaldson's Wistar norm for rats of the same gross body weight than to those of the same length. The values obtained, however, would have been practically the same in either case.

The tables published by Donaldson ('15) used here are designated as 'Donaldson's Wistar tables' or the 'Wistar norms.'

For the dosage employed see table 3.

The experiments began when the rats were weaned, at the age of about 3 weeks, excepting 15 rats which were 8 to 11 weeks old. Of these 15 rats, only 4 were autopsied. Each rat was treated on alternate days throughout the entire period of the experiment. Nine litters were given fresh glandular substance, and all others received dried glands. The material to be fed was mixed with a small amount of bread and milk and the animal kept in an individual cage until all of this had been eaten.

IV. ORIGINAL OBSERVATIONS

1. *Body as a whole* (*weight and length*)

a. Comparison of controls with norms. The growth of the younger groups of rats, especially the males, in this investigation varied considerably from that of the rats described by most of the previous workers for rats collected at random from a colony. It is necessary therefore to make a comparison of the animals with the norms of other investigators before discussing the effects of the ductless gland feeding. In this comparison, only the control rats will be considered directly. With the exception of certain data of the thyroid-fed groups which will be discussed later, however, there is a close agreement among the corresponding data of all the groups so that the data of the control animals represent indirectly most of the data of the entire series.

A great difference between my rats (especially the 'younger' or 'winter-born' males) and those previously described by Donaldson and Jackson is to be noticed in the rate of growth both of body length and of body weight. The albino rats described by Donaldson ('06) and by Jackson ('13 and '15) of different strain and different diet are considerably lighter in weight than are mine at corresponding ages, up to the fourth month. (See table 1 and charts 1 and 2). The selected 'strong and vigorous' litters described by King ('15), however, correspond rather closely with mine in body weight.

The rate of growth varies thus in albino rats from different sources, depending partly upon the 'strain,' but more upon diet and general environment. King's results indicate also that a more rapid growth may be expected from those litters in which at birth the individuals are especially large and strong. The vigorous average growth of my rats appears chiefly in those designated as the 'younger' ('winter-born') litters. The growth of the 'older' ('summer-born') animals throughout is more nearly like that found by Donaldson and Jackson (charts 1 and 2; table 1). As the 'younger' group had been eliminated largely at 110 (and partly at 70) days, the final averages are relatively

TABLE 1

Average gross body-weight of normal albino rats at various ages (in comparison with data from Donaldson, Jackson and King), showing variability probably due to various causes.

AGE DAYS	HOSKINS (CONTROLS)	KING ('15)[1]	JACKSON ('13)	DONALDSON ('06)
	grams	*grams*	*grams*	*grams*
20–21	(11m)[2] 32.6	(50m) 32.0	(53m) 24.0	(19m) 21.2
	(10f) 29.6	(50f) 28.0	(59f) 21.5	(17f) 22.6
30–31	(18m) 46.4	(50m) 48.5		(19m) 31.8
	(16f) 44.4	(50f) 45.7		(17f) 32.9
40	(18m) 69.5			
	(16f) 70.1			
42–43	(18m) 80.9	(50m) 78.0	(45m) 63.7	(19m) 46.3
	(16f) 76.4	(50f) 70.0	(50f) 64.3	(11f) 47.9
70	(18m) 164.2	(50m) 143.0	(23m) 130.4	(19m) 106.6
	(16f) 127.3	(50f) 123.0	(25f) 108.9	(11f) 99.8
90	(15m) 184.6	(50m) 184.8		
	(13f) 143.3	(39f) 148.0		
110–112	(11m) 205.9	(50m) 214.0		(19m) 183.8
	(10f) 151.6	(42f) 166.0		(11f) 160.2
150	(5m) 221.5[3]	(50m) 243.0	(20m) 167.5	(19m) 225.4
	(8f) 164.6	(45f) 185.0	(21f) 142.1	(11f) 184.6

[1] King's data partly from her table 3, partly estimated from her chart 3.

[2] The 21 day group includes only the larger 'winter-born' rats.

[3] The 150 day group includes only the smaller 'summer-born' group.

lower. At 150 days (table 1) the averages are slightly less than Donaldson's and King's, but somewhat greater than Jackson's.

As shown in table 1, the females at six weeks (40 days) average heavier than the males in the control group. This agrees with the results of Donaldson ('06) and Jackson ('13), but not with King ('15).

The difference in the rate of growth of the body length and tail length of my rats, as compared with the data of Jackson ('15) and of Donaldson's Wistar tables is shown in table 2. It may be seen that my rats averaging 13 weeks old are longer than Jackson's rats at 5 to 13 months. Had the table included my entire autopsied series of 59 males and 59 females the difference would have been slightly greater, as seen in tables 6 and 7. The ratio between the tail length and the body length is dif-

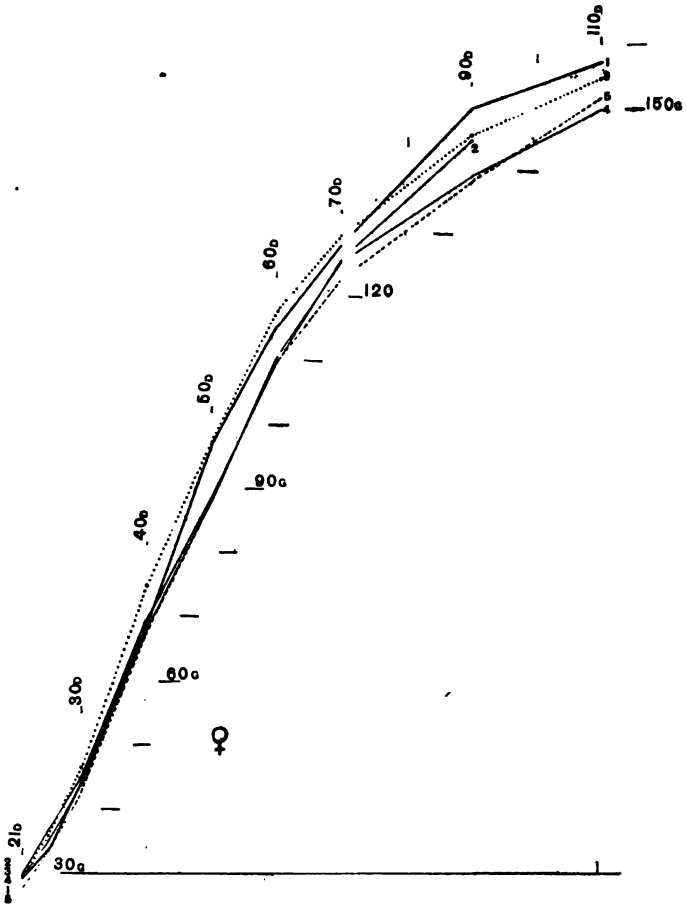

Chart 1 A graphic comparison of the data of various investigators with those of Hoskins for the growth of normal female albino rats. The weight in grams is plotted against the age in days. *D.*, Donaldson '06, *J.*, Jackson '13; *K.*, King '15; *H.*, Hoskins' older (low dosage) group of control female rats and H^1, Hoskins' younger (high dosage) group of control female rats. The sudden flattening of the graphs H^1 and H after 70 and 90 days respectively is due largely to the fact that at these points autopsy of rats was begun. The curves H^1 and H are not closely comparable with the others after these two points, because in most cases the largest rats were killed first.

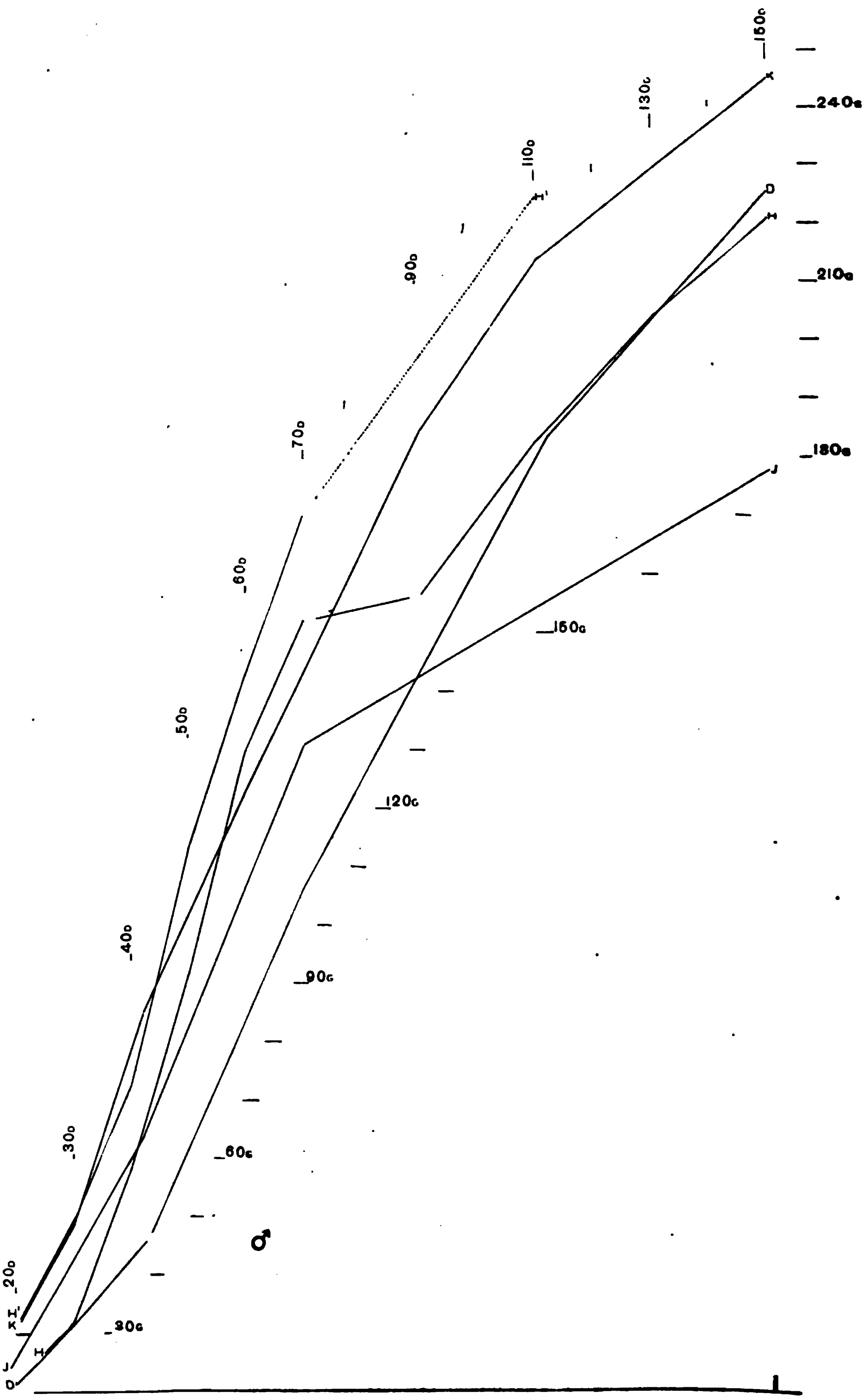

Chart 2 The same as in Chart 1, except that it is for normal males. For further explanation, see that given for chart 1.

TABLE 2

Comparison of data for younger and older groups of control (muscle-fed) albino rats with Jackson's data and Donaldson's tables, showing growth of body length and tail length.

	HOSKINS				JACKSON ('15) 5–13 MO.		DONALDSON'S TABLES (AT CORRESPONDING BODY LENGTHS)	
	Younger group (av. 13 weeks)		Older group (av. 28 weeks)					
Number and sex	8F*	10M*	8F.	6M.	34F.	16M.	F.	M.
Body length, cm	18.7	20.3	19.4	21.2	18.3	19.0	18.7–19.4	20.3–21.2
Tail length, cm	16.0	16.5	16.5	16.6	16.8	16.2	16.4–17.0	17.2–18.0
Tail Body- Ratio	0.86	0.81	0.85	0.78	0.90	0.86	0.88	0.85

* If all the thirteen weeks old rats on experiment are included, the number of females and males changes to 28 and 29 respectively, and the lengths of the body and of the tail are but very slightly changed. (See tables 7 and 8.)

ferent in the different series. In all of my groups of rats the tails are relatively shorter than those of Jackson's rats, and than those at corresponding body lengths in Donaldson's tables. If, instead of the age, the body weight is taken for the basis of comparison, a similar difference in the ratio of the tail length to the body length of the two series is evident.

The growth of the albino rat in weight and length under different circumstances thus varies considerably. A norm must therefore be established not only for each strain but also for each litter under a given set of environmental conditions. If, however, similar litters from the same strain are kept under similar conditions variability will be at a minimum.

For a comparison of the various organs and parts of the rats with those described by previous writers, data are shown in tables 8 and 9 for relative (percentage) weights, and in tables 4, 5, 6 and 7 for absolute weights. Data from Donaldson's Wistar tables for rats of corresponding body weights and lengths are included in tables 4 and 5. The body weight in general is slightly greater in the Wistar norms than in my rats of corresponding body length, excepting the younger males. As to the individual organs, it is evident that in some cases the weights in my series are nearer to those of Donaldson's tables at corresponding body lengths, while in others they are nearer those of corresponding body weight. Some differences are due probably to age. On the whole, the correspondence with the Wistar tables is as close as could be expected. The individual organs will be considered later.

b. Effects of thyroid, thymus, hypophysis and pineal feeding. The weight and length of the growing albino rats fed various ductless glands are shown in tables 6 and 7, and (for the 'higher dosage' groups) in charts 3 and 4. For the sake of elimination of any variation that might be due to the age of the rats, each sex group was subdivided into 2 smaller ('old' and 'young') groups, depending upon the age of the individuals. Some of the younger animals received fresh and some dried glands (see 'Material and Methods'), but no difference was noticed in the effects produced by the two forms.

The effect of a ductless gland diet upon the growth of the females is seen in table 6 and chart 3. The various experimental groups may be compared with each other or with the controls, and it is found that the difference in weight at every age is remarkably slight. At the beginning of the experiment when the rats were 3 weeks old the different groups averaged nearly the same in weight, excepting the male thyroid group and the pineal groups. Into these groups were purposely placed slightly more than their share of smaller animals because it has recently been claimed that thyroid and pineal substances accelerate growth in various species (Schäfer '12) (Dana and Berkeley '13, McCord '14). At 70 days of age, when the period of most rapid growth had ended, it is seen that among the younger ('higher dosage') animals there had been a remarkably small difference in the growth rate of the various groups. The same is true of the older rats, if the thyroid group (which contains 2 rats that were not healthy) is left out of consideration. At 90 days of age the weights of the different groups still remain fairly close together. After 70 days the groups are no longer directly comparable because many of the rats had been killed. Upon the comparison of individuals within each litter, no constant difference appears, although considerable variation is shown. The only probable conclusion to be drawn is that the glandular substances (in

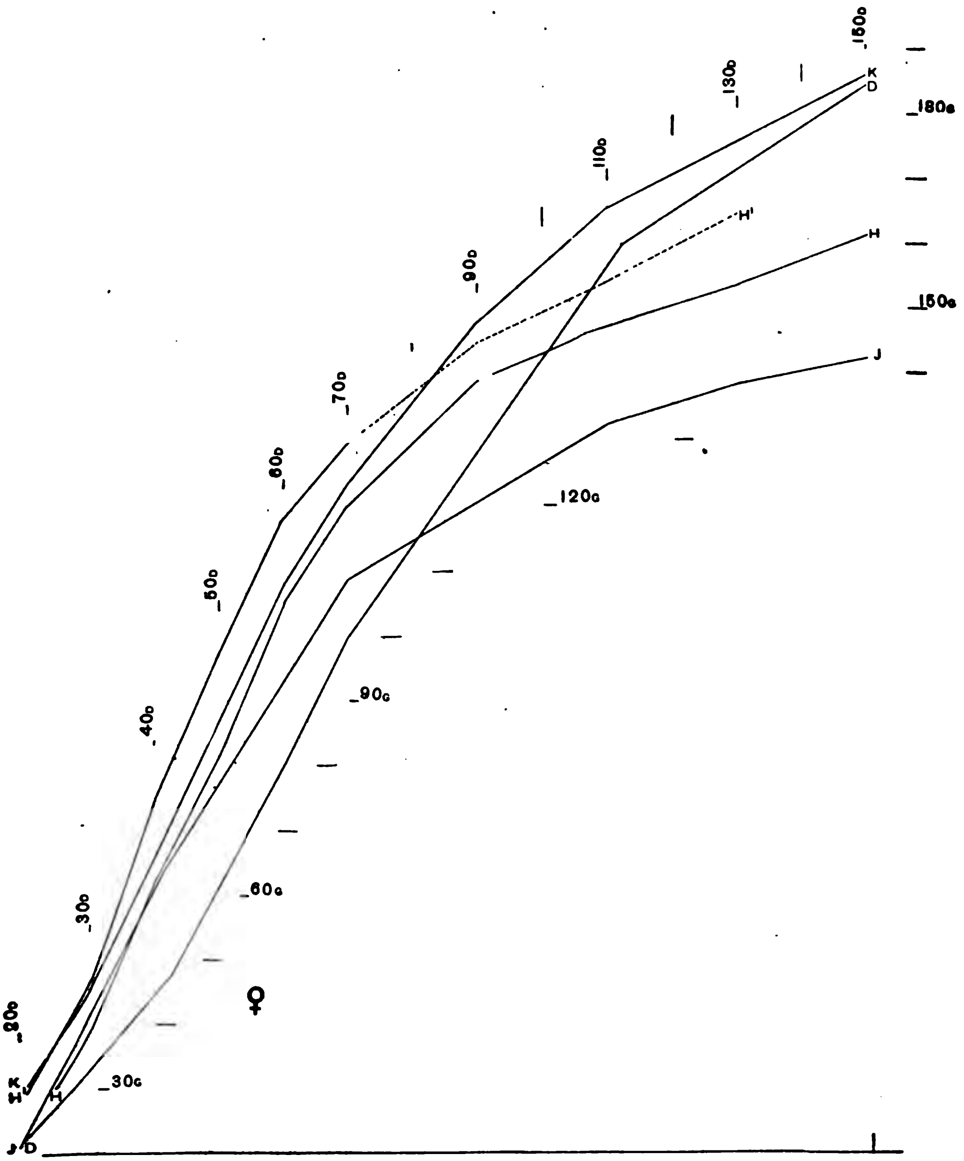

Chart 3 Graphic representation of the growth of the 'younger' or 'high dosage' female albino rats to which ductless glands were fed. The weight in grams is plotted against the age in days. *1*, Thyroid-fed; *2*, Thymus-fed; *3*, Muscle-fed (controls; see also chart 1); *4*, Hypophysis-fed; *5*, Pineal-fed. Note how closely the various groups agree in weight. At 70 days, autopsy of the largest rats was begun, hence the greater variation after this point.

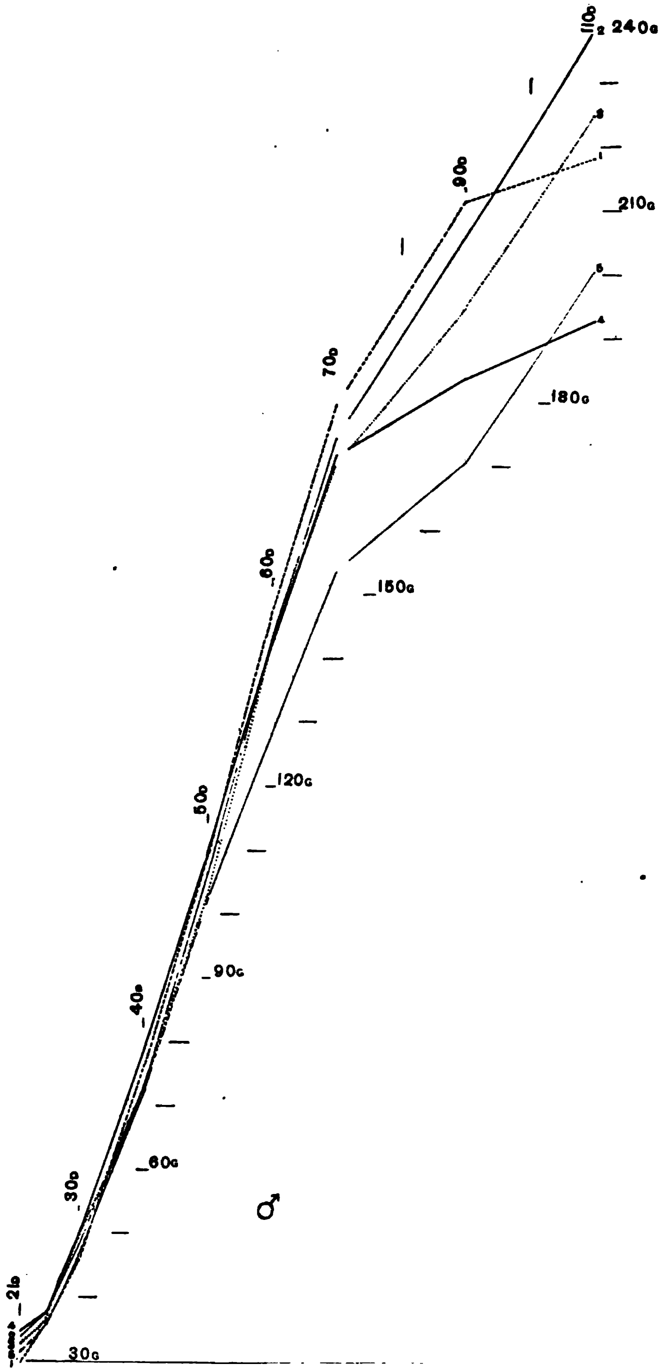

Chart 4 The same as chart 3, except that it is for males. The flattening of the graphs between 21 and 25 days (as compared with those of the females, chart 3) possibly indicates that males are more susceptible to environmental changes than are females. The rats were weaned at 21 days.

the amounts fed) had no effect upon the weight of the female albino rats in this experiment.

A graphic representation of the growth of the 'higher dosage' group of females is shown in chart 3. The body weights in all cases remain close to those of the controls (muscle-fed). In the 'higher dosage' groups, the body weights (chart 1) are seen to be much higher than the normals of Jackson and also of Donaldson (excepting near the end of the experiment). In the 'lower dosage' groups the body weights average lower, more nearly comparable to the normals of Jackson and Donaldson. This difference in body weight between the 'higher dosage' groups and the 'lower dosage' groups is not due to the different amounts of ductless glands fed, however, as a similar difference is shown by the controls in each group. A careful study of the growth of individuals within each litter shows that in nearly every instance those rats which at three weeks were larger (or smaller) than the controls retained the same relative position as regards body weight throughout the experiment.

The male albino rats to which ductless glands were fed also seemed not to be affected in body weight by the treatment. A careful study of table 7 and chart 4 shows nearly the same facts for the male rats as have just been stated for the females. The weights of the different 'higher dosage' groups are unusually close together at 70 days of age, except in the case of the pineal-fed. The 'higher dosage' pineal-fed animals at this age are 16.5 grams lighter in weight than the controls; but this difference is not great, and as this group averaged less in weight than the controls at the beginning of the experiment, the difference in weight between the two is probably due to normal variation. In the 'lower dosage' group, the pineal-fed are slightly above the controls in weight. After 70 days of age many rats were autopsied and hence the groups are no longer directly comparable, but individuals of the same litter were compared with each other and showed the same results as in the case of the females. On the whole, there appears to be no evidence indicating that the ductless gland feeding has naterially affected the body weight in any case. The differences are inconstant, and well within the limits of the variability to be expected.

It may be noted (see table 3) that thyroid was fed in varying amounts from the negligible quantity of 10 mgms. of dried gland on alternate days to a nearly maximum non-toxic dose of 200 mgms. of dried substance (or an equivalent amount of fresh gland) on alternate days. In all cases no appreciable effect upon the growth of the body as a whole was evident. Thymus was fed in variable amounts up to 300 mgms. of dried substance, but two individuals receiving twice this amount showed no effects different from the others. Hypophysis was administered in fairly small doses (5 to 100 mgm. of dried sub-

TABLE 3

Amount and range of dosage employed for each rat in feeding the various groups. The growth in body weight for the 'high dosage' groups is represented in charts 3 and 4.

	SIZE OF SINGLE DOSE		DOSAGE EMPLOYED FOR			
			Females		Males	
	Dried substance	Fresh substance	Average number of doses	Range	Average number of doses	Range
	mgms.	*mgms.*				
Thyroid-fed:	10	40				
'Low dosage'			4.3	4–5	1.6	0.5–2
'High dosage'			11.7	10–20	16.3	10–20
Thymus-fed:	15	70				
'Low dosage'			3.5	2–5	1.0	0.5–2
'High dosage'			16.7	10–40	16.4	10–20
Muscle-fed (controls):	8	25				
'Low dosage'			3.8	2–5	2.4	0.5–5
'High dosage'			15.8	10–40	17.0	10–30
Hypophysis-fed:	5	25				
'Low dosage'			3.5	2–5	2.3	0.5–5
'High dosage'			12.5	10–20	18.6	10–30
Pineal-fed:	7.5	40				
'Low dosage'			3.8	2–5	2.0	0.5–3
'High dosage'			17.5	10–40	13.3	10–20

stance). Pineal substance was given in amounts larger than those fed to guinea pigs (in most cases) by McCord ('14), but in one of McCord's experiments where doses of 100 mgms. of dried substance was used daily, the experimental animals in a given time grew in weight 40 per cent more than the controls. As stated above, there seemed to be no difference in the result with albino rats in the present experiment, whether small amounts of 20 mgms. or larger amounts up to 150 mgms. of dried pineal substance was fed on alternate days. With the larger doses there was no evidence indicating that toxic effects of gastrointestinal disturbances were produced by the medication.

As shown in tables 2, 6 and 7, the various rats of the same age and sex are also of nearly the same body-length regardless of treatment. The agreement here is even closer than in regard to comparative weights and emphasizes still more strongly the fact that the experimental rats as compared with the controls suffered no marked gross body changes on account of the administration of ductless glands.

The growth records of the individual rats illustrate, as has been pointed out above, that very serious errors might easily creep into the conclusions from an investigation of this kind, which includes animals from several different litters. A preliminary comparison of the individuals in each litter showed negative results, so all were finally grouped as shown in the various tables. There is always, of course, some danger in drawing conclusions from averages, but this danger is slight if the individual data are also carefully studied, and the grouping judiciously made.

Care must be taken in work of this nature to select experimental and control animals from the same litter and as nearly alike as possible. In many investigations on growth this has not been done. Experimental groups of animals of one sex have even been compared with groups of another sex; or, more commonly, an experimental group has contained both sexes in a ratio different from that in the control group.

Finally, the depressing effects upon growth and body weight obtained by some investigators by the administration of ductless glands especially the thyroid (Magnus-Levy, Bircher, Carlson, Farrant, Gudernatsch, Cotroni, Hewitt, Romeis, Lenhart), is in many cases possibly a general toxic effect, which is produced whenever the dosage is too high. Even a high protein diet, e.g., an excessive meat diet, may likewise be detrimental to growth, as has been shown for the albino rat by Chalmers Watson ('06).

2. Head

Data for percentage weights of head (and other organs and parts) are found for females in table 8, and for males in table 9. Absolute weights are given in tables 6 and 7.

a. Controls. The head forms an average of 9.5 per cent of the body weight in the females and 8.3 per cent in the males among my younger control rats. In the older groups, the head averages 10.3 per cent in the females, and 8.4 per cent in the males. These results are in general somewhat lower than those obtained by Jackson ('13, '15).

b. Thyroid group. The head averages very slightly heavier in both females and males to which thyroid was fed. The difference is probably insignificant.

c. Thymus, hypophysis and pineal groups. The head in these groups shows no constant variation from the controls in either direction. The few small differences are probably not significant.

3. Eviscerated Body (*Tables 6 to 9*)

a. Controls. The eviscerated body forms an average of 80 to 84 per cent of the net body weight in both males and females. This part of the body contains the muscles, skin, skeleton, body-fat, great vessels, lymph nodes, and spinal cord.

b. Thyroid group. The eviscerated body in all rats of both sexes (excepting 3 old males, in which the dosage was slight) is about 4 per cent less in relative (percentage) weight than that of the controls. This loss is due probably to loss of fat, which is a well-known effect of thyroid-feeding, especially with high dosage. A comparison of the body weights and body lengths in the control (muscle-fed) and thyroid groups shows a slight relative decrease in weight in the thyroid males of higher dosage, but not in the females.

c. Thymus, hypophysis and pineal groups. In all these groups, the weight of the eviscerated body is very close to that of the controls (muscle-fed). The variations are slight and probably insignificant.

4. Integument (tables 6 to 9)

a. Controls. The average relative (percentage) weight of the integument is relatively fairly close in the various groups of both sexes. In general, the percentages range between 21 and 24 per cent of the net body weight, the value for the male rats being slightly greater than for the females, due possibly to the presence of a greater amount of fat in the latter. This is somewhat higher than that observed by Jackson and Lowrey ('12), by more than 4 per cent of the entire net body weight, and is 2 per cent of the entire body weight higher than that observed by Jackson ('15). These differences are probably due largely to the varying amount of fat (or muscle) present in the integument.

b. Thyroid group. In the thyroid rats of each sex the integument appears usually very slightly lighter in weight than in the controls, owing probably to loss of fat. Jackson ('15) has shown that during inanition the skin loses greatly in weight (probably due chiefly to loss of fat) but that the relative (percentage) weight remains unchanged in adults.

c. Thymus, hypophysis and pineal groups. In these groups, the differences in weight of the integument, as compared within each group and with the controls (muscle-fed), are well within the limits of normal variation.

5. Cartilaginous skeleton (tables 6 to 9)

a. Controls. The relative weight of the 'wet' cartilaginous skeleton averages about 7.6 per cent of the body weight in the older females, and about 6.6 per cent in the older males. The skeleton in the younger females averages about 6.8 per cent of the net body weight and in the younger males about 6.1 per cent. These correspond fairly well with the estimate of Jackson ('15) which was 7 per cent of the adult net body weight, and with Conrow's observations (cited by Donaldson '15, table 53). The difference between the sexes is accounted for by the heavier body weight (with correspondingly lighter skeleton) in the male groups, rather than by any true sexual difference.

The older groups of each sex on the average appear to have a relatively heavier skeleton than the younger. This is contrary to the general tendency of the skeleton during growth to lag behind in relative weight. In these groups, however, the differences in body weight are much less than usual for the corresponding age differences, and the increased weight in the older skeletons is possibly due to more advanced stages of ossification and calcification. Thus in two animals of the same body weight, the older apparently has a heavier skeleton. This tendency is not evident in Conrow's data (cited by Donaldson '15), however.

In this connection may be cited the observations of Jackson ('15) who found that in young rats held at constant body weight by underfeeding the skeleton continues its development (differentiation and increase in wet and dry weights). It is therefore probable that the relative weight of the skeleton depends somewhat upon the age factor, as skeletal growth is to some extent independent of the general growth of the body.

The dry cartilaginous skeleton is likewise relatively slightly heavier in the female (3.7 to 4.6 per cent) than in the male (3.2 to 3.8 per cent), and the older rats of both sexes have relatively heavier dry skeletons than the younger. The explanation for this is doubtless the same as that above given for similar relations in the weights of the wet skeleton in different groups. There is considerable variation shown by individuals, so that the average values for both wet and dry skeleton can be considered as only approximate. Differences in the technique may also modify the skeletal weight considerably. For example, Conrow's data for the dry skeleton (cited in Donaldson's table 53) are too high, because her method of drying at room temperature is inadequate to remove all the moisture. More data are needed to establish a satisfactory norm.

b. Thyroid groups. The skeleton appears slightly heavier for the thyroid treatment in the average of the younger groups. A comparison of the individual data shows that both wet and dry skeletons average somewhat heavier in most of the rats receiving higher dosage of thyroid than in those of low dosage and controls. This suggests that the thyroid treatment may perhaps tend to stimulate skeletal development, as claimed by Bircher ('10b). However, the possibility of errors from accidental variations must be kept in mind, as the differences found are not very great, and were not constant in every litter.

c. Thymus, hypophysis, and pineal groups. The various groups fed thymus, hypophysis and pineal glands show no important or constant variation from the controls in regard to the skeleton. If any effect was produced, it is so slight as to be masked by normal variation.

6. Brain (tables 4 to 10)

a. Controls. As shown by tables 4 and 5, the absolute weight of the brain does not vary greatly from that shown by Donaldson's tables for rats of similar sex and body weight or body length.

In percentage weights, as might be expected, the males have relatively lighter brains than the females, and the older brains average lighter than the younger, on account of the fact that the brain lags behind in the growth of the body.

b. Thyroid groups. On comparing directly the average relative (percentage) weights (tables 8 and 9) of the controls (muscle-fed) and thyroid-fed animals, the brain in the latter appears slightly heavier in all except the younger group of males; but on comparing the absolute weights with the Wistar norms for animals of corresponding body weight, it is found that the difference in the various litters is not constant and the brain of the thyroid-fed rats averages even smaller than the normal (table 10).

c. Thymus, hypophysis, and pineal groups. There is no constant variation of the brain in these groups. There appears to be a slight tendency to increase in the brains of the hypophysis-fed group (less marked than in the case of the thyroid), but the difference is not constant.

7. *Eyeballs (tables 4 to 10)*

a. Controls. The absolute weight of the eyeballs in my animals corresponds fairly closely to that of Donaldson's norms (excepting the younger males), as shown in tables 4 and 5. In comparing the relative weights of groups of each sex (tables 8 and 9) the eyeballs appear relatively heavier in the older groups, although in these, with heavier body weight, the eyeballs would be expected to be relatively lighter. As suggested by Jackson

TABLE 4

Comparison of principal data for control female albino rats with the Wistar tables of Donaldson ('15). Weight is the average expressed in grams. Comparison is made with Wistar norms both of the same body length and of the same gross body weight.

	HOSKINS (OLDER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WT.)	HOSKINS (YOUNGER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)
Body length, cm	19.4	19.4	18.9	18.7	18.7	18.5
Gross body weight, g.	171.8	188.5	172.6	159.8	166.6	160.8
Tail-Ratio	0.85	0.88		0.86	0.88	
Age (days)	213			92		
Organs:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Brain	1.781	1.823	1.801	1.782	1.791	1.782
Eyeballs	0.276	0.263	0.252	0.223	0.248	0.244
Heart	0.737	0.765	0.715	0.694	0.696	0.677
Kidneys	1.379	1.622	1.503	1.589	1.458	1.414
Liver	7.530	9.600	9.000	7.405	8.770	8.540
Spleen	0.751	0.505	0.465	0.611	0.450	0.435
Lungs	1.900	1.119	1.040	0.982	1.010	0.981
Alimentary Tract	6.966	9.680	9.120			
Ovaries	0.053	0.049	0.048	0.064	0.048	0.047
Hypophysis	0.011	0.013	0.012	0.010	0.011	0.011
Suprarenals	0.049	0.050	0.046	0.049	0.045	0.044
Thymus*	(0.134)	(0.153)		(0.264)	(0.278)	

* Thymus compared with regard to age (213 days average for the older group, and 92 days for the younger group) instead of regard to body length or weight.

TABLE 5

Comparison of principal data for control male albino rats with the Wistar Tables of Donaldson ('15). Weight is the average expressed in grams. Comparison is made with the Wistar norms of the same body length and of the same gross body weight.

	HOSKINS (OLDER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)	HOSKINS (YOUNGER)	WISTAR (SAME LENGTH)	WISTAR (SAME BODY WEIGHT)
Body length, cm	21.2	21.2	21.1	20.3	20.3	20.6
Gross body weight, g.	232.5	238.1	234.1	214.9	204.3	215.0
Tail-Ratio	0.783	0.85	0.85	0.813	0.85	0.85
Age (days)	193			90		
Organs:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Brain	1.852	1.911	1.907	1.909	1.872	1.885
Eyeballs	0.286	0.294	0.291	0.220	0.273	0.280
Heart	0.893	0.918	0.905	0.988	0.814	0.847
Kidneys	1.867	1.992	1.962	2.050	1.740	1.820
Liver	10.701	11.440	11.290	10.915	10.200	10.590
Spleen	0.959	0.630	0.620	0.895	0.545	0.572
Lungs	1.813	1.365	1.346	1.199	1.198	1.251
Alimentary Tract	9.351	11.360	11.230			
Testes	2.222	2.397	2.375	2.248	2.203	2.267
Hypophysis	0.009	0.009	0.008	0.008	0.008	0.008
Suprarenals	0.031	0.038	0.038	0.031	0.035	0.036
Thymus*	(0.155)	(0.170)		(0.298)	(0.283)	

* Thymus compared with regard to age (193 days average for older group, and 90 days average for younger group) instead of regard to body length or weight.

('13), however, the growth of the eyeballs may tend to be correlated with age, rather than with body weight (as is known to occur in the thymus). These differences may therefore be due to age changes, to normal variability, or perhaps merely to differences in the technique of removal of the eyeballs.

b. Thyroid groups. The eyeballs average heavier in relative weight than those of the controls in both sexes, but the differences obtained are slight and probably of no significance. Comparison according to the method of Donaldson ('15) shows them slightly lighter in weight in the males than those of the corresponding controls, but in the females slightly heavier.

c. Thymus, hypophysis and pineal groups. In most cases, the eyeballs in these groups average relatively slightly heavier than those of the controls. No particular significance other than variability is attached to this fact.

8. *Thyroid Gland* (*tables 6 to 9*)

a. Controls. The thyroid of my rats cannot be compared directly with that of other investigators owing to the different technique with which it was removed from the body, as described in 'Material and Methods.' The weight is about one-third less than the Wistar norm, probably on account of removal of the capsule. There is also extreme variation in the weight of this gland even in different members of the same litter. Usually it appears relatively heavier in the older than in the younger rats, and also slightly heavier in females. Jackson '(13) and Hatai ('13) also have found the thyroid gland to be exceedingly variable, so no final conclusions can be drawn as to the normal weight of this gland.

b. Thyroid groups. The thyroid gland (tables 6 to 9) shows no constant changes as a result of the thyroid feeding. Any effect if produced is hidden by the great normal variability. In view of the great variability in the weight of this organ, final conclusions in regard to the effect of thyroid feeding upon the weight of the thyroid gland are not justified from the available observations.

c. Thymus, hypophysis, and pineal groups. In these groups likewise the thyroid appears variable when compared with the controls, and the results are not sufficiently marked or constant to warrant any conclusion regarding the effects upon the weight of the thyroid gland of feeding these substances.

9. *Thymus* (*tables 4 to 9*)

a. Controls. The thymus also is not to be compared very closely with that described by previous workers. The usual method of comparison considers the weight of the gland at different ages. Theoretically, the organ in the albino rat increases in size gradually until at about 85 days it reaches its maximum weight of 0.29 grams (Hatai '14), but as is well known, many conditions influence the involution of this organ. Its weight varies considerably in rats of the same size and age even when the animals are apparently normal and kept under nearly

TABLE 6

Females: Average absolute weight (in grams) of parts in albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	5	4	5	6	8	8	6	5	7	5
Average age (days)	207	80	215	88	213	92	196	92	214	95
Gross body weight, g.	160.2	145.3	158.0	156.3	171.9	159.9	166.1	149.6	173.3	146.2
Net body weight, g.	169.7	140.2	151.3	149.1	165.4	152.9	151.8	142.4	164.7	138.3
Body length, cm	19.3	18.4	18.9	18.5	19.4	18.7	18.8	18.6	19.5	18.3
Tail length, cm	15.9	16.0	16.4	15.6	16.5	16.0	15.3	16.0	15.9	15.6
Average number doses	8.6	12.5	11.7	16.7	11.6	16.3	12.8	12.0	11.9	20.0
Average weight of:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Head	15.6	13.4	15.8	13.9	16.3	1.41	15.6	14.4	16.3	14.0
Eviscerated body	127.6	125.7	123.5	135.8	148.5	128.7	128.4	126.4	143.9	117.9
Integument	31.5	32.5	32.4	33.9	36.3	31.7	31.2	32.0	35.3	29.2
Wet cartilaginous skeleton	11.24	9.95	10.88	7.94	11.18	10.39	11.94	10.22	12.05	10.40
Dry cartilaginous skeleton	6.69	5.49	6.63	5.23	7.47	5.51	7.02	5.64	7.332	5.623
Brain	1.714	1.723	1.683	1.742	1.781	1.782	1.714	1.806	1.858	1.538
Eyeballs	0.276	0.211	0.275	0.177	0.276	0.223	0.276	0.228	0.282	2.27
Thyroid	0.017	0.14	0.014	0.018	0.017	0.015	0.0197	0.0151	0.018	0.017
Thymus	0.120	0.232	0.074	0.221	0.134	0.264	0.125	0.220	0.114	0.212
Heart	0.850	0.761	0.672	0.656	0.737	0.694	0.663	0.676	0.696	0.611
Lungs	1.917	0.851	1.588	0.945	1.900	0.982	2.047	0.911	1.929	1.208
Liver	9.383	9.266	6.943	8.567	7.530	7.405	6.941	7.484	7.180	8.188
Spleen	0.910	0.633	0.794	0.537	0.751	0.611	0.633	0.534	0.502	0.659
Stomach-intestines (empty)	7.445	3.172[1]	6.528	3.137	6.966	2.962[1]	7.191	3.244[1]	7.568	3.690
Suprarenals	0.0647	0.0525	0.0393	0.0454	0.0485	0.0486	0.0493	0.0548	0.0464	0.0395
Kidneys	1.916	1.829	1.620	1.574	1.379	1.589	1.289	1.641	1.485	1.337
Ovaries	0.496	0.652	0.436	0.614	0.053	0.064	0.530	0.615	0.455	0.492
Pineal	0.0011	0.0012	0.0011	0.0012	0.0011	0.0011	0.0012	0.0011	0.0011	0.0012
Hypophysis	0.0093	0.0088	0.0101	0.0088	0.0111	0.0100	0.0101	0.0094	0.0098	0.0084

[1] Freed of mesenteries and pancreas.

TABLE 7

Males: Average absolute weight (in grams) of parts of albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	7	4	5	4	6	10	6	7	6	4
Average age (days)	164	94	162	94	193	90	188	84	202	87
Gross body weight	210.5	209.5	225.6	231.9	235.0	214.9	228.9	208.7	233.6	192.7
Net body weight	198.9	199.3	215.0	223.8	225.3	206.3	215.4	197.5	227.3	184.8
Body length, cm	21.0	20.7	21.2	20.7	21.2	20.3	21.0	19.7	21.2	19.4
Tail length, cm	17.0	17.3	16.8	17.2	16.6	16.5	16.8	16.9	17.0	15.9
Average number doses	10.8	15.0	11.7	17.5	10.0	17.0	10.3	20.7	7.4	15.0
Average weight of:	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Head	17.2	16.9	17.9	17.6	18.8	17.3	18.7	16.0	18.9	15.3
Eviscerated body		166.4	169.8	191.9	184.9	171.6	177.6	156.7	205.2	159.5
Integument	43.3	46.2	47.6	52.0	51.3	47.2	46.2	53.7	51.9	43.4
Wet cartilaginous skeleton	13.36	12.42	15.43	13.10	14.62	12.77	13.67	11.43	14.13	14.37
Dry cartilaginous skeleton	7.93	6.82	8.72	7.20	8.59	6.70	8.44	6.02	8.66	6.07
Brain	1.839	1.850	1.872	1.887	1.852	1.909	1.846	1.800	1.802	1.729
Eyeballs	0.269	0.226	0.283	0.225	0.281	0.220	0.277	0.210	0.227	0.208
Thyroid	0.021	0.014	0.023	0.016	0.028	0.016	0.023	0.020	0.023	0.021
Thymus	0.164	0.298	0.220	0.333	0.155	0.298	0.153	0.323	0.139	0.225
Heart	1.060	1.119	0.988	0.957	0.893	0.988	0.896	0.851	0.857	0.824
Lungs	1.768	1.522	1.996	1.458	1.665	1.199	1.954	1.091	1.008	1.402
Liver	11.563	12.183	10.182	11.063	10.701	10.991	10.991	10.763	11.999	9.208
Spleen	1.241	0.997	0.796	0.559	0.958	0.895	0.883	0.868	0.709	0.717
Stomach-intestines (empty)	9.496	3.179[1]	9.533	3.740[1]	9.351	4.097[1]	8.392	3.408[1]	9.479	3.941[1]
Suprarenals	0.0361	0.0469	0.0340	0.0356	0.0306	0.0311	0.0281	0.0309	0.0302	0.0263
Kidneys	2.223	2.387	1.894	2.086	1.867	2.050	1.891	1.979	2.162	1.676
Testes	2.148	2.422	2.121	2.274	2.222	2.248	2.047	1.903	2.450	2.031
Epididymi	0.748	0.644	0.794	0.624	0.753	0.613	0.672	0.547	0.736	0.539
Pineal	0.0013	0.0018	0.0015	0.0013	0.0014	0.0014	0.0014	0.0012	0.0012	0.0014
Hypophysis	0.0080	0.0090	0.0081	0 0083	0.0085	0.0079	0.0082	0.0073	0.0078	0.007

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[1] Freed of mesenteries and pancreas.

TABLE 8

Females: Average relative (percentage of net body) weight of parts in albino rats grouped according to diet and age when autopsied

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	5	4	5	6	8	8	6	5	7	5
Average age (days)	207	80.5	215.4	87.5	212.6	91.7	195.6	91.8	213.7	95.4
Average net body weight g	169.7	140.2	151.3	149.1	165.4	152.9	151.8	142.4	164.7	138.0
Average number dose	8.6	12.5	11.4	16.7	11.6	16.3	12.8	12.0	11.9	20.0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Head	10.56	9.64	10.61	9.34	10.28	9.54	10.28	10.13	10.00	10.02
Eviscerated body	79.26	80.87	82.08	83.15	83.72	82.51	82.44	83.69	83.33	82.02
Integument	21.04	22.95	21.36	22.86	21.75	22.43	20.89	22.55	21.35	21.15
Wet cartilaginous skeleton	7.53	7.12	7.31	6.86	7.57	6.81	7.61	7.15	7.44	7.31
Dry cartilaginous skeleton	4.46	3.92	4.45	3.53	4.59	3.69	4.45	4.00	4.47	3.93
Brain	1.16	1.26	1.14	1.19	1.10	1.17	1.14	1.27	1.05	1.23
Eyeballs	0.187	0.152	0.186	0.149	0.171	0.141	0.188	0.162	0.172	0.164
Thyroid	0.011	0.010	0.010	0.012	0.011	0.010	0.013	0.010	0.011	0.012
Thymus	0.080	0.161	0.061	0.157	0.070	0.172	0.079	0.184	0.071	0.154
Heart	0.576	0.546	0.446	0.444	0.449	0.455	4.43	.0.477	0.428	0.442
Lungs	1.31	0.61	1.03	0.62	1.22	0.64	1.40	0.64	1.04	0.87
Liver	6.24	5.98	4.63	5.71	4.58	4.89	4.13	5.30	4.36	5.87
Spleen	0.604	0.473	0.530	0.361	0.478	0.400	0.418	0.373	0.438	.481
Stomach-intestines (empty)	4.96	2.32[1]	4.59	2.49[1]	4.24	2.07[1]	4.79	2.36[1]	4.62	2.73[1]
Suprarenals	0.043	0.037	0.026	0.031	0.029	0.031	0.030	0.035	0.029	0.028
Kidneys	1.30	1.32	1.06	1.06	0.92	1.04	1.05	1.09	0.91	1.05
Ovaries	0.032	0.047	0.029	0.041	0.032	0.043	0.034	0.039	0.029	0.036
Pineal	0.00075	0.00084	0.00073	0.00084	0.00067	0.00069	0.00080	0.00078	0.00075	0.00082
Hypophysis	0.0062	0.0063	0.0068	0.0062	0.0067	0.0065	0.0067	0.0067	0.0059	0.0061

[1] Freed of mesenteries and pancreas.

TABLE 9

Males: Average relative (percentage of net body) weight of parts in rats grouped according to diet and age when killed

	THYROID-FED		THYMUS-FED		MUSCLE-FED (CONTROLS)		HYPOPHYSIS-FED		PINEAL-FED	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Number of rats	7	4	5	4	6	10	6	7	6	4
Average age (days)	164	93.8	161.6	94.3	192.9	90.0	188.1	84	202.1	87
Average net body weight g	198.9	200.3	215.0	227.5	225.3	206.3	215.4	197.5	227.0	184.8
Average number doses	10.8	15.0	11.7	17.5	10.0	17.0	10.3	20.7	7.4	15.0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Head	7.37	8.44	8.38	7.89	8.40	8.34	8.84	8.13	8.46	7.89
Eviscerated body	83.1	80.74	82.85	84.24	82.24	83.20	81.81	80.07	83.46	83.52
Integument	21.33	22.86	22.05	22.95	22.48	23.02	21.65	23.35	21.94	23.51
Wet cartilaginous skeleton	6.52	6.23	7.03	5.90	6.57	6.13	6.20	5.97	6.24	6.35
Dry cartilaginous skeleton	4.02[2]	3.39	4.09	3.31	3.83	3.21	3.89	3.06	3.96	3.28
Brain	0.93	0.93	0.88	0.86	0.84	0.94	0.88	0.91	0.80	0.94
Eyeballs	0.129	0.114	0.130	0.102	0.119	0.110	0.122	0.110	0.110	0.113
Thyroid	0.010	0.007	0.011	0.007	0.013	0.008	0.011	0.010	0.012	0.011
Thymus	0.73	0.155	0.104	0.153	0.072	0.051	0.072	0.167	0.62	0.167
Heart	0.545	0.555	0.465	0.486	0.401	0.472	0.425	0.435	0.435	0.446
Lungs	0.89	0.76	0.94	0.65	0.74	0.60	0.92	0.55	1.01	0.79
Liver	5.88	6.16	4.76	5.04	4.87	5.16	5.23	5.47	4.13	4.95
Spleen	0.634	0.516	0.398	0.257	0.453	0.443	0.437	0.439	0.315	0.419
Stomach-intestines (empty)	4.95	2.23[1]	4.43	1.88[1]	4.18	2.19	4.05	2.07[1]	4.05	2.14[1]
Suprarenals	0.019	0.024	0.016	0.016	0.014	0.015	0.013	0.016	0.014	0.016
Kidneys	1.13	1.18	0.89	0.94	0.91	1.05	0.89	1.01	0.82	0.90
Testes	1.08	1.20	0.98	1.06	1.01	1.09	0.98	0.96	0.95	1.09
Epididymi	0.38	0.31	0.37	0.28	0.34	0.36	0.32	0.28	0.35	0.29
Pineal	0.0065	0.00088	0.00070	0.00056	0.00062	0.00069	0.00065	0.00061	0.00052	0.00076
Hypophysis	0.044	0.0045	0.0038	0.0037	0.0038	0.0039	0.0037	0.0037	0.0034	0.0040

[1] Freed of mesenteries and pancreas.

[2] Group contains 1 rat with an exceptionally heavy skeleton.

identical conditions. According to Jackson ('13) the coefficient of variability in weight of the thymus at 10 weeks is 22 to 25.

When compared in absolute weight with Donaldson's Wistar norm for rats of corresponding age (tables 4 and 5) it appears that in my rats the thymus is somewhat lighter, except in the younger male group. The age involution is well known on comparing the older and younger groups (tables 6 to 9), the corresponding body weights in both cases being not greatly different.

b. Thyroid groups. The thymus shows no constant difference in weight that can be attributed to the thyroid diet. It is under weight in the females by an average of 10 per cent, but not in the males.

c. Thymus, hypophysis, and pineal groups. The thymus appears relatively much heavier than normal in the thymus-fed older males, but as no corresponding difference is found in the younger males, or in the females, the result is probably due to accidental variations.

10. Heart (tables 4 to 10)

a. Controls. In absolute weight, the heart of the older rats in my series (tables 4 and 5) is in general in fairly close agreement with the corresponding data in Donaldson's tables, but in my younger males the heart is considerably heavier than Donaldson's norm for rats of corresponding body weight or length. Jackson ('13) also found the normal heart relatively somewhat heavier than would be expected according to Hatai's curve of theoretical growth.

b. Thyroid groups. The heart shows a very marked hypertrophy in rats to which thyroid was fed, excepting 3 old males, in which the dosage was too small to be effective. If calculated by Donaldson's method, the hypertrophy amounts to 24.6 and 16.7 per cent for the older and younger females respectively, and 36 and 15.4 per cent for the corresponding males (table 10).

c. Thymus, hypophysis, and pineal groups. No constant or apparently significant variations appear in the heart in these groups.

TABLE 10

Thyroid-fed albino rats. Average percentage deviation of organ weight from that of the control rats, compared according to Donaldson's method ('The Rat,' Donaldson '15).

ORGAN	OLDER GROUPS		YOUNGER GROUPS	
	Females	Males	Females	Males
	per cent	*per cent*	*per cent*	*per cent*
Brain	− 3.6	+ 0.1	+ 0.5	− 5.5
Eyeballs	+ 0.5	− 0.1	+ 6.8	− 8.5
Heart	+24.6	+36.0	+16.7	+15.4
Liver	+26.7	+24.6	+30.5	+ 6.4
Spleen	+41.3	+86.0	+15.0	+ 6.4
Alimentary canal	+12.1	+11.8		
Suprarenals	+16.1	+38.1	+14.5	+36.4
Kidneys	+46.3	+44.4	+33.0	+40.4
Ovaries	− 3.2		− 3.5	
Testes		− 0.2		+ 9.8
Hypophysis	−11.5	+21.3	− 8.8	+18.0

11. Lungs (tables 4 to 9)

a. Controls. The weights of the lungs in the older rats are considerably heavier than those of Donaldson's tables, owing to the prevalence of pulmonary infection in my older rats. The younger rats are in close agreement with the Wistar tables.

b. Thyroid groups. On account of the great variation due to the frequency of lung infection (especially in the older animals), no conclusions can be drawn from the apparent changes in the weight of the lungs in the thyroid-fed groups.

c. Thymus, hypophysis, and pineal groups. There are no differences that are of significance in the weights of the lungs in these groups.

12. Liver (tables 4 to 10)

a. Controls. In absolute weight, the liver averaged somewhat below the corresponding figures in Donaldson's Wistar tables, excepting in the younger males. Jackson ('13) also found the liver normally considerably below the theoretical curve derived by Hatai ('13). The liver is known to vary greatly under different circumstances, however, and is considerably influenced in size by diet, etc. One might expect a relatively larger liver in the female, which is said to be more active than the male (Slonaker '12), since exercise has been shown to cause hypertrophy of this organ (Hatai '15). Jackson ('13) found the liver heavier in the male, however, and this is apparently true also for my control animals.

b. Thyroid groups. In the thyroid-fed groups, (tables 8 to 10) the liver appears relatively considerably heavier than in the controls. When compared according to Donaldson's method, the females show an increase in the absolute weight of the liver of 26.7 per cent and 30.5 per cent for the older and younger groups respectively, and the males show a corresponding increase of 24.4 per cent and 6.4 per cent. The apparently small increase in the weight of the liver of the younger males is probably to be explained by the fact that the liver is unusually heavy in the corresponding control group. Even when due allowance for normal variability is made, a hypertrophy of the liver due to thyroid feeding is therefore strongly indicated.

c. Thymus, hypophysis, and pineal groups. In all the younger groups of females the liver averages larger than in the controls, but as no corresponding difference is found in the other groups, the variation is probably not significant.

13. Spleen (tables 4 to 10)

a. Controls. As appears by comparing the individual data, the absolute weight of the spleen in the control groups is from 30 per cent to 100 per cent higher than the figures for corresponding animals in Donaldson's Wistar tables. In the latter tables, however, the figures for the spleen are derived from the formula of Hatai ('13) who used data from which the 'enlarged' spleens had been excluded. Whether these tables for the spleen really represent the true norm is therefore questionable, since it is not known whether the 'enlarged' spleens are actually pathological, or represent merely extreme cases of normal variation in size. Jackson ('13) found the variability of the spleen in the rat very high (coefficient of variation averaged about 35). His relative (percentage) values for the normal spleen (including all specimens) are also somewhat above the curve derived from Hatai's formula (excluding enlarged specimens), but are considerably lower than mine, excepting the old male group (tables 8 and 9). It is evident that my control spleens are relatively larger than that which is usually considered normal, but it is impossible to say whether or not this is due to normal variability. A study of individual data shows that the weight of the spleen in most rats varies in the same direction as that of the liver. Dr. Hatai of the Wistar Institute has told me that he also has noted such correlation. This fact is in agreement with the doctrine that the spleen functions in furnishing certain materials to the liver for use in general metabolism. Sweet and Ellis ('15) state that where digestion is interfered with greatly by removal of the external function of the pancreas, the spleen undergoes marked simple atrophy.

b. Thyroid groups (tables 6 to 10). In relative weight where the averages of the thyroid groups are compared with the entire control group it is seen that there is an increase in the weight of the spleen of about 25 per cent. According to Donaldson's method of comparison the increase in absolute weight amounts to 41.3 per cent and 15 per cent in the older and younger female groups, and of 86 per cent and 6.4 per cent in the corresponding male groups (table 10). The apparently small increase shown by the younger group of males is due to an unusually large spleen in the control group, in which the liver was also unusually large (as above mentioned).

c. Thymus, hypophysis, and pineal groups. As might be expected, the spleen in these groups shows considerable variability, but it is probably within the limits of normal variation. There appear decidedly smaller spleens in the thymus-fed male group, but the results are not constant, and of doubtful significance.

14. Alimentary canal (tables 4 to 10)

a. Controls. As may be seen in tables 4 and 5, the absolute weights obtained for the alimentary canal (empty stomach and intestines, plus mesentery and pancreas) average slightly lower than in Donaldson's tables corresponding to the older males and females. In my younger rats, no data for this system are available. The alimentary canal, including the stomach and intestines when freed from the pancreas and mesentery, weighs about 3 grams in the younger female rats (average 13 weeks old) and 4.1 grams in the males of the same age, forming about 2.1 per cent of the net body weight in the former and a little less in the latter (tables 8 and 9). The measurements can be made only approximately on account of the difficulty in removing completely the contents of the canal without loss of a part of the mucosa. No data for comparison with the digestive canal without mesentery are available.

b. Thyroid groups. The empty alimentary canal appears heavier in relative weight both in males and females of the older thyroid groups than in the corresponding controls (tables 8 and 9). In no case is the difference very large, however, and owing to variability and difficulty in securing exact weights of the empty canal, the difference is of doubtful significance. Compared by Donaldson's method, the increase in weight is about 12 per cent in each older group (table 10).

c. Thymus, hypophysis, and pineal groups. The variations observed in the alimentary canal of these groups (tables 6 to 9) in comparison with the controls are inconstant, and probably within the limits of normal variability and experimental error.

15. Suprarenal glands (tables 4 to 10)

a. Controls. In absolute weight as compared with Donaldson's norms, the suprarenals of my rats (tables 4 and 5) are somewhat light in the case of the males, but correspond more closely in the females.

In relative weight (tables 8 and 9) my data correspond fairly well with the results of Jackson ('13), but the suprarenals of my rats have a slightly higher percentage in the females and lower in the males, than in Jackson's older rats of corresponding body weight. The sexual difference in the weight of the suprarenals discovered independently by Jackson ('13) and by Hatai ('13) occurs likewise in my rats.

b. Thyroid groups. As shown in tables 6 to 10, the thyroid-fed animals show a distinct increase in the relative size of the suprarenals in all groups. According to Donaldson's method of comparison, the younger females show an overgrowth in the absolute weight of the suprarenals of 14.5 per cent, the older females of 16.1 per cent, the younger males of 36.4 per cent, and the older males of 38.1 per cent (table 10). This indicates that the reaction of the suprarenals to thyroid treatment is relatively greater in males, in which sex the gland is normally relatively lighter in weight than it is in the females.

c. Thymus, hypophysis, and pineal groups. In all these groups the suprarenal glands do not appear to differ from the controls more than might be expected from the normal variability (tables 6 to 9). In the younger pineal-fed female rats, however, the suprarenals average about 9 per cent lighter in weight than in the corresponding controls.

16. Kidneys (tables 4 to 10)

a. Controls. As compared with Donaldson's tables, the kidneys in my rats (tables 4 and 5) were somewhat heavier in the younger groups and lighter in the older groups. The differences are not great, however, and may possibly be due to age. My younger rats are larger than is usual at that age. The relative size of the kidney tends to decrease with the less active metabolism of adult life.

In relative (percentage) weight (tables 8 and 9), all the groups are in fairly close agreement with the results obtained by Jackson ('13) for rats of the same age. In two rats of the same sex and same size but of different age, the younger usually has larger kidneys.

b. Thyroid groups. The kidneys reacted to the thyroid feeding in a manner similar to that described for the liver, but to a greater extent (tables 6 to 10). According to Donaldson's method of comparison the increase in the absolute weight of the kidneys is as follows: older females 46.3 per cent, younger females 33.0 per cent, older males 44.4 per cent, and younger males 40.4 per cent (table 10).

c. Thymus, hypophysis and pineal groups. As shown in tables 6 to 9, the kidneys in the various groups fed on thymus, hypophysis and pineal gland show no very constant variations from the controls. The differences are mostly small and are probably due to normal variability. The kidneys of the hypophysis-fed females average 12 per cent heavier than the controls, however, and those of the pineal-fed males average 16 per cent under normal weight.

17. Ovaries (tables 4, 6, 8 and 10)

a. Controls. In absolute weight, the ovaries appear considerably heavier for the younger group, and slightly heavier for the older group (table 4) than the corresponding ovaries in Donaldson's tables. In relative (percentage) weight, the ovary in the younger groups similarly exceeds the normal found by Jackson ('13), and is about equal in the older group. The ovaries are normally found to be exceedingly variable in weight. This is to be explained partly on account of varying stages of ovulation, and partly to technical difficulties of dissection (Jackson '13).

b. Thyroid groups. The ovaries in the thyroid groups appear nearly unchanged, the slight differences being very probably due to normal variation.

c. Thymus, hypophysis, and pineal groups. On comparing the relative weights of the ovaries in these groups, no important or constant changes are found (table 8), except in the pineal-fed group. In these the ovaries appear considerably under weight, as compared with the controls. The decrease in absolute weight shown by Donaldson's method is 16.7 and 27.0

per cent for the older and younger groups respectively. Possibly pineal feeding retards growth of ovaries, but on account of their great variability and the comparatively small number of observations, there is considerable doubt as to whether the decrease noted is significant.

18. *Testes (tables 5, 7, 9 and 10)*

a. Controls. In absolute weight, compared with Donaldson's tables, the testes in my rats average slightly lighter (table 5) In relative (percentage) weight (table 9), in comparison with the observations of Jackson ('13) on animals of corresponding weight, the testes in my series appear relatively lighter in the older group and of about the same relative weight in the younger groups. Jackson's data included the epididymis, however, and on this basis, with testis and epididymis combined, both my old and young groups would appear to have heavier organs.

b. Thyroid group. The testes of both sub-groups of the thyroid-fed males (table 9) show a slight increase in relative weight as compared with the entire group of controls. This overgrowth ranges from about 7 per cent in the group of older rats (in part of which the dosage is very small and ineffective)to 13 per cent in the young animals. Calculated by Donaldson's method the older rats are seen to have testes of the same absolute weight as the controls, and the younger rats to have heavier (10 per cent) testes than the corresponding controls. As in the case of the ovaries, however, the testes show considerable normal variability, and conclusions based upon a relatively small number of observations must be carefully guarded.

c. Thymus, hypophysis, and pineal groups. In these groups the testes show no important or constant changes in comparison with the controls, and the differences (e.g., the apparent slight retardation of the testis in pineal-fed) are probably not significant. Microscopic examination of the testis of a thymus-fed rat showed no degeneration, although Hewer ('14) found such condition in rats, some of which were fed but slightly more thymus than some of my rats received. (Mine were fed up to 1.4 grams fresh or 0.15 grams dried thymus on alternate days; hers were fed 1 to 4 grams of the fresh thymus daily.)

19. *Epididymi* (*tables 7 and 9*)

a. Controls. Few data are available for comparison. Jackson ('13) states that after the age of puberty the epididymis weighs about one-third as much as the testis, and in extreme cases one-half. In my series (tables 7 and 9) the eipdidymi average about one-fourth as heavy as the testes in the younger (0.28 per cent of net body weight), and about one-third as heavy in the older group (0.34 per cent of net body weight). Some of the variability in the weight of the epididymis is due to the difficulty in removing the fat associated with it.

b. Thyroid groups. The epididymi in the thyroid group, like the testes, appear slightly larger than in the control rats (tables 7 and 9). It is doubtful whether they are stimulated in growth by the thyroid treatment, however.

c. Thymus, hypophysis, and pineal groups. In these groups, as was true in regard to the testis, the variations in comparison with the controls are probably not significant.

20. *Pineal body* (*tables 6 to 9*)

a. Controls. No attempt has hitherto been made to determine the normal weight of this small organ in a large series of albino rats. Biedl ('13) gives the weight as 0.0020 g., which is considerably higher than the average weights in my series (0.0011 g. in the females and 0.0013–0.0014 g. in the males, tables 6 and 7). The weight values obtained vary greatly on account of the extremely small size of the gland. Relatively (tables 8 and 9) there seems to be no significant difference according to sex or age, although the percentage weight appears to be somewhat less in the animals of greater body weight, and this to some extent is due to age. Recently, from data secured through the kindness of Dr. Donaldson and Dr. Hatai, I note that the values obtained for the pineal body in rats at the Wistar Institute agree fairly closely with mine.

b. Thyroid groups. The pineal body in the thryoid-fed rats (tables 8 and 9) averages in relative weight somewhat heavier in the various groups than in the corresponding controls. The difference is not constant in every litter so that, although it is possible that the thyroid medication increases the weight of this organ, the data are insufficient for a final conclusion.

c. Thymus, hypophysis, and pineal groups. On comparing the relative weights of the pineal body in these groups, the results in the case of the thymus-fed and hypophysis-fed are very inconstant. In the pineal-fed, as in the thyroid-fed rats, the pineal body appears usually increased in relative weight. The difference in weight is not constant in every litter, and hence is of doubtful significance, in all of these three groups.

21. Hypophysis (tables 7 to 10)

a. Controls. In absolute weight the hypophysis in both male and female rats in my series agree fairly well with Donaldson's tables for rats of corresponding body length or body weight (tables 4 and 5). There is a sexual difference in the weight of this organ, as discovered by Hatai ('13). As seen in tables 8 and 9, the average percentage of the net body weight for the hypophysis is about 0.0066 per cent in the females and 0.0039 per cent in the males (not much change according to age or body weight). Thus the hypophysis, like the suprarenal body, is in the adult rats relatively much larger in the female.

b. Thyroid groups. On comparing the thyroid-fed animals with the controls (tables 8 to 10), there appears a very slight decrease in relative weight of the hypophysis in the female groups. In both male groups there is a decided increase in the weight of the hypophysis. With Donaldson's method of comparison the decrease in absolute weight in the older and younger female groups is 11.5 and 8.8 per cent respectively, and the increase in the corresponding males is 21.3 and 18.0 per cent (table 10). These results may be interpreted as indicating a sexual difference in the effect of thyroid feeding upon the hypophysis, the effect being most marked in the males. The thyroid-feeding therefore tends to reduce the normal difference between the sexes in the weight of the hypophysis.

22. Summary

a. Comparison of controls with established norms for body weight, body and tail length, and size of organs. In regard to body weight, the control rats used in this investigation as well as the experimental animals can be divided into two groups: (1) an older group ('summer-born') which were born in the summer and early fall; and (2) a younger group ('winter-born') which were born in the winter and spring. The first group maintained about the same size and body weight as the rats of Donaldson ('06) and Jackson ('13). The second group, especially the males, were considerably larger at every stage, and more nearly resembled in growth the selected strong and vigorous litters of rats recently studied by King ('15).

The ratio of tail length to body length is somewhat less in my series than in the norms of Jackson ('15) and those calculated from Donaldson's Wistar tables. That is, my rats were relatively short-tailed.

The variability of growth in body weight, body length and tail length is thus emphasized. Such differences may occur in different 'strains' of rats, especially under different conditions of environment, diet, etc. Experience shows that the average body weight may be considerably increased by unusual care and liberal feeding. Exercise has also been shown to affect markedly the growth of the body (Slonaker '12) and organs (Hatai '15) of the rat.

As to the absolute weights of the individual organs and parts, the data for the controls in general agree fairly well with those of Donaldson's Wistar tables for corresponding body length and body weight (tables 4 and 5). The differences are in most instances no greater than might be expected from normal variability, especially in rats of different strain kept under different environment. In some cases (e.g., thyroid gland, thymus, and alimentary canal) there are differences due to difference in the technique of removing the organs.

In the case of the very rapidly growing rats, it is quite probable that some of the peculiarities (e.g., of skeleton, spleen, liver, heart, kidneys) in comparison with older rats of similar body weight may be due to changes with age, independent of body weight. The younger rats with more rapid metabolism may have relatively larger organs. Donaldson has found a close correlation between the water content of the central nervous system and age in the rat. The age involution of the thymus is well known, and Jackson ('13) found indications of correlation with age in the weight of the eyeballs. Similar relations are possible in other organs, although organ weight in general is doubtless more closely correlated with body weight than with age.

In relative (percentage) weights, the organs likewise are in general correspondence with the results of Jackson ('15), the differences in most cases being within the limits of normal variability to be expected. There was noted a marked correlation between the weights of the spleen and liver in most cases.

b. Effects of thyroid feeding. The thyroid diet apparently affected the gross body weight of the rats but very slightly. The 'higher dosage' animals averaged slightly heavier than the controls and other groups (charts 3 and 4), but the difference is perhaps too slight to be significant. If the loss in fat of the thyroid-fed animals be taken into account, an increased weight of the remainder of the body appears.

The results are not incompatible with the view that by thyroid feeding with small dosage an increase in body weight is produced (as found by Moussu '99, and Schäfer '12), while with higher dosage a decrease or retardation in body weight is produced (Magnus-Levy '95; Moussu '99, Bircher '10; Carlson, Rooke and McKie '12; Gudernatsch '12, '14, '15; Hewitt' 14; Cotroni '14; Romeis '15). The decrease or retardation of body weight reported by most of the above mentioned investigators is possibly due to the toxic effect of large doses. I observed that two thyroid-fed rats apparently in good health were killed by a single large dose (200 mgm.) of dried thyroid substance.

The eviscerated body and (to a slighter extent) the integument of the thyroid-fed fats usually show a slight loss in relative weight, probably due to loss of fat.

The organs of the rats which received very little thyroid were not much affected in weight, but in the rats which received larger doses (see tables 6 to 10) several of the organs are evidently hypertrophied. This cannot be interpreted as merely a relative gain, due to loss of body-fat, for the body weight, as above stated, has apparently suffered no loss in the thyroid-fed rats, but possibly a slight gain instead. The organs which show a considerable increase in weight are the heart, liver, spleen, suprarenals and kidneys.

Other organs in which the results are more doubtful, but which seem to be increased in weight to a greater or lesser extent are the hypophysis (male), and alimentary canal, and possibly the skeleton, testes, and epididymi. The increase of this group of organs is uncertain, and in some cases probably due to chance variations.

These results are in general agreement with those of Iscovesco ('13) who (by thyroid extract injections) found hypertrophy of the heart, thyroid gland, suprarenals, kidneys (male), testes, ovaries and uterus. He reports an increase in the weight of these organs of nearly 100 per cent, but no increase in the weight of the liver or female kidneys. My results confirm also the hypertrophy of the suprarenal glands found by Rudinger, Falta and Eppinger ('08), and by Hoskins ('10a). They also confirm to some extent the results of Bircher ('10a) (10b), who found that hyperthyroidism produces enlargement of the heart and thyroid gland, and acceleration of skeletal development. They do not, however, agree with Utterström ('10), who found enlargement of the thymus.

It may be noted further that these results likewise agree with the generally accepted doctrine that hyperthyroidism produces a general acceleration of metabolism. Conditions increasing metabolism would naturally throw a greater burden upon the important viscera, and thus tend to produce in them a hypertrophy. Thus Hatai ('15) has shown that increased exercise in albino rats tends to produce an enlargement of various organs, and his results resemble closely those obtained by me with thyroid feeding.

c. Effects of thymus feeding. In general, the results of thymus feeding were negative. Neither the body as a whole nor any of the individual parts or organs showed any marked or constant apparent effect, in comparison with the controls. The apparent tendency to decrease in the relative weight of the male spleen is a doubtful exception.

The negative results are in agreement with those of Miss Hewer ('14), except as to the degeneration of the testis; but they do not confirm in the rat the stimulating effect upon body growth obtained by Gudernatsch ('12) ('14) and (in part) by Romeis ('15) in amphibian larvae.

d. Effects of hypophysis feeding. The data in the present experiments show that the weights of the body as a whole and of the various parts and organs of the albino rats in the hypophysis-fed groups were even more closely in agreement with the controls than were the thymus groups. The apparent increase in weight shown by the kidneys and thyroid of the females is probably not significant.

The negative results of the hypophysis feeding in these experiments confirm similar findings as to negative effects upon body weight by Caselli ('00); Sandri (anterior lobe)('09); Hoskins ('11); Aldrich ('12a) ('12b); Schäfer ('12); Lewis and Miller ('13), and Gudernatsch ('14). On the other hand they do not agree with Schäfer ('09) and Goetsch ('16) who from very few data reported growth stimulated by feeding anterior lobe of hypophysis, nor with the following who obtained retardation of growth (especially skeletal) by hypophysis feeding: Thompson and Johnston ('05); Etienne and Parisot ('08); Sandri ('07) ('09) (posterior lobe); Cushing and Goetsch (cited by Cushing '12); Wulzen ('14), and Pearl ('16) (anterior lobe). My results likewise disagree with Hallion and Alquier ('08) who obtained hypertrophy of the suprarenal glands, and with Wulzen ('14) who found accelerated involution of the thymus. It may be noted, however, that my dosage was comparatively small subtoxic, and this might account for the negative results.

e. Effects of pineal feeding. The pineal groups also agree fairly closely in weight with the control animals, both as to the body as a whole and in the various organs and parts. Several minor differences are to be noted, but all are perhaps within the limit of normal variability. The testes, kidneys (male), ovaries, and suprarenals show a decrease in weight which is of doubtful significance, as (except in the case of the ovaries) it is not constant in every litter. My results agree with Priore ('15) who obtained negative results on body growth by injection of pineal extracts. They disagree, however, with those of Dana and Berkeley ('14) and McCord ('14, '15) who obtained an acceleration in body growth by feeding pineal substance.

V. CONCLUSIONS

Some of the principal results of the present investigation may be summed up briefly as follows:

1. The normal growth of the albino rat varies materially, not only in different 'strains' and under different conditions of environment, but even among litters when all conditions are as nearly constant as possible. It is therefore not sufficient to rely upon the established norms of growth for comparison, but in all cases experimental and control animals should be selected of the same sex and from the same litter or litters.

2. In the case of rats with unusually rapid (or slow) growth, in addition to comparison with (older or younger) rats of corresponding size and weight, due regard for possible changes correlated with age must be observed. Such changes have previously been noted in the water content of nervous system (Donaldson), weight of the thymus (Hatai), and probably the weight of the eyeballs (Jackson). Indications of similar age-changes (independent of body weight) are found in the skeleton, liver, kidneys, heart and spleen. Compared with animals of the usual age at a given body weight, the skeleton seems to be relatively heavy in older animals, and the other organs are apparently heavier in younger rapidly growing animals.

3. There is apparently no sexual difference in the relative weight of the pineal body, such as has been found in the suprarenal glands (Jackson '13, Hatai '13) and the hypophysis (Hatai '13).

4. Thyroid feeding (in sub-toxic doses) causes little or no change in body weight in growing rats. There is possibly a slight stimulation in growth of the body as a whole, balanced by a decrease in the amount of free fat in the body. There is a slight loss in the relative weights of the eviscerated body and the integument, probably due to loss of body-fat.

5. Thyroid feeding produces a decided hypertrophy of the heart, liver, spleen, kidneys and suprarenal glands (especially in males). It apparently causes also a somewhat less extensive and more uncertain increase in the weights of the alimentary canal and hypophysis (male) and possibly in the skeleton, testes and epididymi, and a decrease in the weight of the hypophysis of females.

6. Thymus feeding (with the dosage employed) has no apparent effect upon the growth rate of the body of albino rats. No constant or important effect upon any of the individual organs or parts was observed. The testis showed no degenerative or other changes.

7. Hypophysis feeding (with the sub-toxic dosage employed) produces no marked or constant effect upon the growth rate of the body or organs of albino rats.

8. Pineal feeding likewise produces no apparent changes in the weight of the body or organs of the albino rat, beyond differences probably within the limits of normal variation.

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EFFECTS OF INANITION UPON THE STRUCTURE OF THE THYROID AND PARATHYROID GLANDS OF THE ALBINO RAT

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INTRODUCTION

The thyroid gland presents an interesting and difficult biological problem. Although the morphology of the thyroid has been extensively studied, there are still many doubtful and unsettled questions concerning its development and its normal adult structure. Even more uncertainty exists concerning its physiological significance and its pathological changes. Some light may be thrown upon the various phases of this problem by a study of the changes produced in the thyroid gland by inanition.

In previous papers (Jackson '15 a, '15 b) it was shown that during inanition the various organs of the albino rat suffer very unequally in loss of weight, the loss also varying according to the length and character of the inanition. In acute inanition of adult rats, with loss of about one-third in body weight, the thyroid gland had apparently lost but little if any in absolute weight; in chronic inanition of adults, the average apparent loss of the thyroid was about 22 per cent; while in young rats held at maintenance (constant body weight) by underfeeding for several weeks the average loss was about 24 per cent. In order to determine what histological changes are correlated with these changes in gross weight, material was preserved for further study. On account of its intimate association with the thyroid, the parathyroid gland was also included in this investigation. The results are presented in the present paper, which is the third of a series of studies upon the effects of inanition. The work is being carried on with the assistance of a special grant from the research fund of the Graduate School of the University of Minnesota.

MATERIAL AND METHODS

The material used included the thyroid (and included parathyroid) glands of the albino rat (Mus norvegicus albinus) from previous studies (Jackson '15 a, '15 b), together with some collected since. In all, more than 50 normal glands were sectioned and studied, varying in age from newborn to adult (15 months). These were chiefly controls from the same litters as those used for experiments (including several controls used in experiments by E. R. Hoskins and C. A. Stewart). In addition, I am indebted to Professor Bensley and Mr. Burgett, of the University of Chicago, Professor Addison, of the University of Pennsylvania, and Professor Evans, of the University of California, for material kindly furnished in order to investigate possible local variations in normal thyroid structure of the rat.

Of the animals subjected to inanition, the thyroid and parathyroid were obtained from 14 of the younger rats held at maintenance for various periods (chiefly beginning at 3 weeks and ending at 10 weeks of age). Of the adult rats, 6 glands were studied from those subjected to acute inanition, and 3 from those with chronic inanition.

The material was obtained at the autopsy held immediately after the animals were killed, and was fixed chiefly in Zenker's fluid, 12 to 24 hours. In a few cases formalin or Flemming's fluid was used, but the results were less satisfactory.

The glands were embedded in paraffin, and cut at 5 micra (occasionally 7 to 10 micra) in thickness. In the great majority of cases, the glands were cut and mounted in complete serial sections. This was found to be important, not only to make certain of including the parathyroids, but also because the structure frequently varies in different parts of the thyroid and parathyroid glands.

The sections were stained in most cases with haematoxylin and eosin; in a few cases with iron-haematoxylin, safranin, Mallory's anilin-blue connective tissue stain, etc.

All of the drawings were made with a Zeiss 2 mm. 1.30 N. A. apochromatic objective and compensating ocular No. 6, with the aid of a camera lucida. A wheel-micrometer eyepiece was used for the measurements. It was the original intention to measure systematically a large number of cells and nuclei in the glands of the controls and of the animals subjected to inanition. On account of the great irregularity in the size and shape of cells and nuclei, however, it was found that the results, though of limited value, could not be obtained with sufficient accuracy to justify any very extensive series of observations. Therefore the number of measurements was restricted to that judged sufficient to give merely an approximation of the apparent average and range observed.

THE THYROID GLAND

The normal structure of the thyroid gland in the albino rat at various ages will first be considered. Then the changes found in the various types of inanition will be described and their significance discussed.

a. Normal structure of the thyroid gland

The general form and topography of the thyroid gland in the albino rat is shown in cross section in figure 1. Each lateral lobe presents the typical relations—convex external surface covered by the infrahyoid musculature; concave internal surface in contact with the lower larynx and upper trachea; and narrower posterior surface (or border) in relation with the oesophagus medially the carotid artery, etc., laterally. The isthmus is frequently a very thin somewhat fibrous band, almost invisible when the fresh gland is exposed in situ. It invariably contains thyroid follicles (contrary to Sobotta '15), although these may become scattered and more or less atrophied in adult rats.

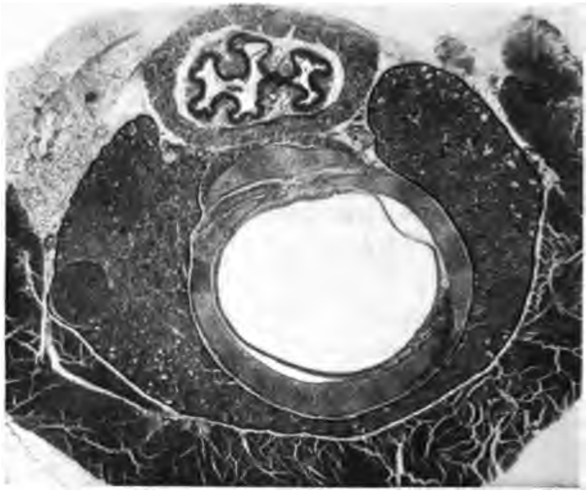


Fig. 1 From a photograph (retouched) of a cross section of the thyroid gland in situ at the level of the isthmus, showing relations to infrahyoid musculature, uppermost trachea, oesophagus, etc. One parathyroid is visible, on the left side of the figure. From albino rat No. S 9.47, age 22 days, gross body-weight 25.5 grams. (× 28.)

The minute structure of the normal thyroid gland at 3 weeks (the age when the experiments began with the younger rats) is shown with slight magnification in figure 1, and under high power in figure 2. No attempt will be made to describe in detail the minute structure of the gland, but some of the essential features especially affected by inanition will be considered briefly.

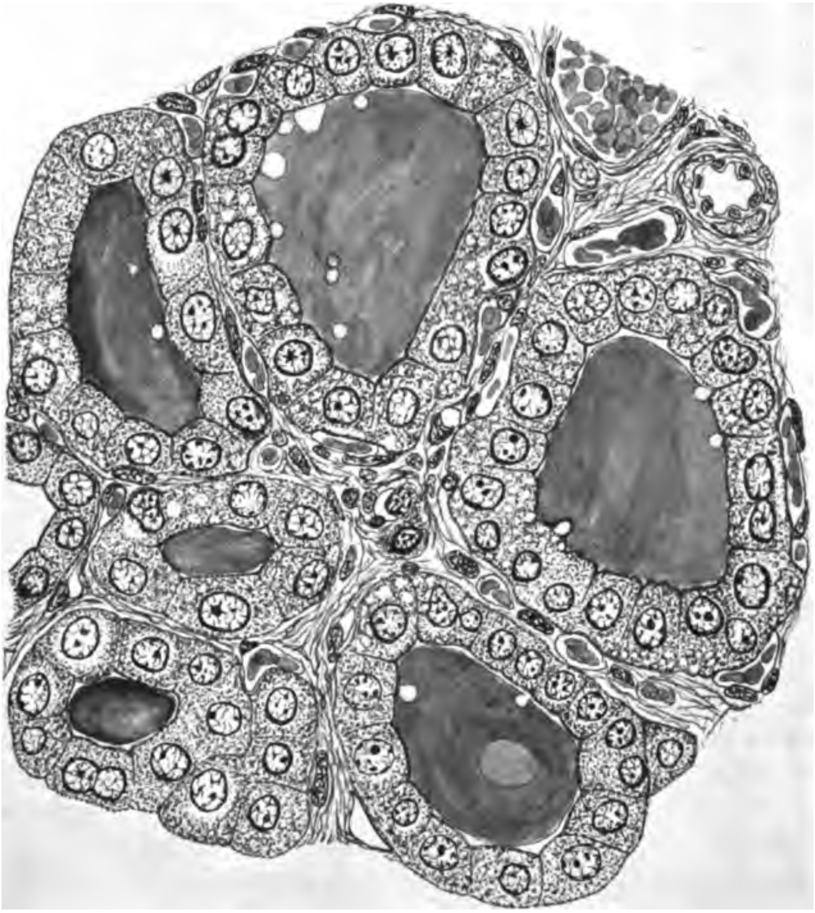


Fig. 2 A small portion of the thyroid gland shown in figure 1 (rat S 9.47, age 22 days) magnified to show the details of the normal histological structure. Several follicles containing colloid are shown. Follicular epithelium cuobidal; cytoplasm abundant and granular, with a few scattered vacuoles. Apparent origin of the colloid 'vacuoles' from the epithelial cells is shown in a few places. Four interfollicular epithelial cells are seen between the two lower follicles. Fibrous stroma scanty, with rich blood-vascular plexus. (× 750.)

The follicles at this time are chiefly oval or rounded in outline and vary mostly from 20 to 70 micra in diameter. The larger follicles are rather infrequent and somewhat uniformly scattered, but are usually more frequent near the surface of the gland. Those shown in figure 2 are of average size.

The finer structure of the thyroid gland is shown in figure 2. The cells of the follicular epithelium are approximately cuboidal in form (in some cases low columnar; in others, especially in the larger peripheral follicles, somewhat flattened). In height, they range chiefly between 8 and 15 micra, the average being 10 to 12 micra. The inner and outer cell walls are sharply distinct; the intercellular boundaries are less distinct and sometimes absent.

The cytoplasm of the follicular cells (fig. 2) is filled with moderately fine granules, reddish violet in color (with Zenker fixation and haematoxylin-eosin stain). The granules are usually somewhat uniformly distributed. They are not densely packed, but are sometimes arranged so as to give an indefinite reticular form, apparently intermingled with small, clear vacuoles. Some of these vacuoles (though not all) may correspond to the minute fat droplets or granules described in the thyroid cells by Erdheim ('03) and Traina ('04). The cells present a fairly uniform appearance, and there is nothing to indicate any division into the 'chief' and 'colloid' cell types of Langendorff.

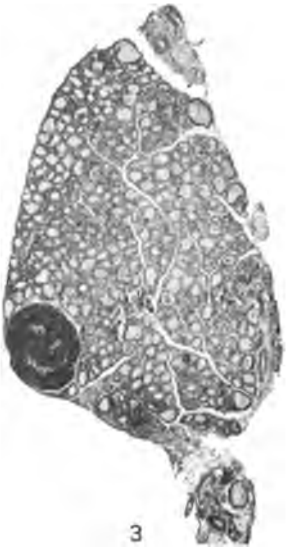
The nuclei of the follicular cells are spherical or slightly ovoidal (ellipsoidal) in form, the diameters varying from 4 to 7 micra, usually 5 or 6 micra. The nuclear membrane is distinct and stains deeply. There are usually one or two larger nucleoli (karyosomes) and several smaller granules; and a fine, paler nuclear network, often indistinct, with a very pale bluish, homogeneous nuclear background, corresponding to the nuclear sap (karyolymph). The nuclei shown in figure 2 are typical, though in the larger peripheral follicles with slightly flattened cells the nuclei may also be somewhat more flattened and slightly hyperchromatic. Cells in mitosis are relatively frequent, 5 having been noted in one entire cross-section of one lobe, and 6 or 8 in another.

The colloid (fig. 2) appears typical in form though somewhat variable in staining reactions. In some cases it fills the follicular cavity completely, in other cases it is retracted somewhat with either smooth or serrated margin. This retraction is probably an artefact in most cases, due to shrinkage produced by the reagents used. But I cannot agree with those investigators who explain the vacuoles (some of which are shown in figure 2) in a similar manner. These vacuoles are usually small (4 micra or less) and spherical in form, and are most frequent near the surface of the colloid. Occasionally they are found in intimate relation with the adjacent cells, from which they are apparently extruded, as shown in the follicle on the right in figure 2. They are probably connected in some way with the process of colloid formation, as described by Anderson ('94) and Müller ('96). Desquamated epithelial cells, which sometimes dissolve leaving clear vacuoles in the colloid in older rats, are extremely rare at this stage.

A variable amount of interfollicular epithelium appears, which cannot be distinguished from tangential sections of follicles, except in serial sections. In structure, these interstitial epithelial cells are similar to those of the follicles. A few appear in the lower part of figure 2.

The interfollicular connective tissue forms a delicate fibrous stroma (fig. 2), relatively small in amount, but containing a rich capillary plexus of blood-vessels. The nuclei visible are mostly of capillary endothelium. They are elongated or flattened in form, and stain somewhat deeply.

At 10 weeks (the age when the inanition experiments ended in most of the younger rats) the thyroid gland has normally undergone but slight changes, the structure being essentially the same as that just described at three weeks. Therefore no detailed figures are considered necessary. A photograph with low magnification is shown in figure 3, representing a cross section of one lateral lobe. The follicles have increased somewhat in size, the maximum diameter now reaching about 100 micra. The larger follicles are more frequent, and are sometimes rather uniformly distributed, as shown in figure 3, though very frequently



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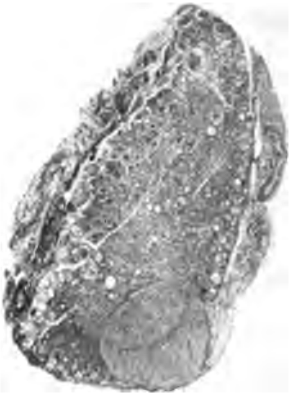

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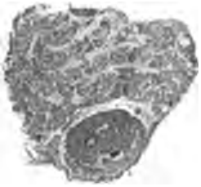

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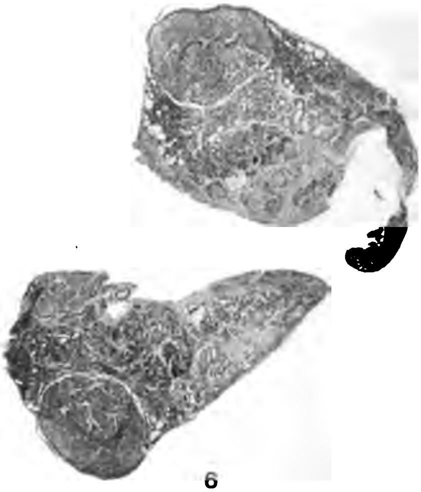

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Fig. 3 From a photograph of one lobe of the thyroid gland from normal albino rat No. S 5.3, age 74 days, gross body-weight 172 grams. Parathyroid included. Compare with figures 4, 5 and 6, representing thyroids in rats of the same age, but held at maintenance from age of 3 weeks. (× 28.)

Fig. 4 From a photograph of one lobe of the thyroid gland from albino rat No. S 11.63, age 72 days, gross body-weight 23.8 grams (held at maintenance from age of 3 weeks). Follicles much larger at periphery. Some extra-capsular tissue is included. Parathyroid relatively large. (× 28.)

there is a distinct tendency to larger follicles in the superficial layers.

In finer structure, the thyroid at 10 weeks is very similar to that shown at 3 weeks (fig. 2). The height of the follicular cells varies considerably, however. While the maximum is about the same as at 3 weeks, the average (about 8 to 10 micra) is somewhat lower. In other words, the cells are usually more flattened, especially toward the periphery of the gland. In the larger surface follicles, the cells are usually distinctly flattened, 6 micra or less in height. The nuclei in these cells are also correspondingly flattened, their diameters averaging 4 x 6 micra. The nuclei in general are similar to those at 3 weeks in size and structure, usually nearly spherical in form, and averaging about 6 micra in diameter. Mitosis is very much less frequent in the cells of the thyroid at 10 weeks than was found in the gland at 3 weeks.

While the typical normal structure of the thyroid cells at 10 weeks is like that at 3 weeks (fig. 2), cells of abnormal appearance are also found. These atypical forms vary from slight modifications up to marked cellular degenerations, and should be carefully noted in order to avoid confusion with the changes during inanition to be described later.

The flattening of the epithelium in the larger peripheral follicles is almost constant, though variable in extent. The flattened nuclei are hyperchromatic in type, and often present a more or less deeply-staining, homogeneous background, which may obscure or obliterate the nuclear network and granules. The cytoplasm is reduced in amount, more deeply-staining, and often somewhat homogeneous in appearance. This type of cell occurs so constantly in the peripheral follicles (and occasionally elsewhere) that it can hardly be considered abnormal. I inter-

Fig. 5 From a photograph of one lobe of the thyroid gland from albino rat No. S 5.10, age 67 days, gross body-weight 22.7 grams (held at maintenance from age of 3 weeks). Gland small, with relative increase of stroma. Parathyroid included. (× 28.)

Fig. 6 From a photograph of both lobes and a portion of the isthmus of the thyroid gland from albino rat No. S 11.64, age 73 days, gross body-weight 24.2 grams (held at maintenance from age of 3 weeks). Follicles larger at periphery but irregular, many degenerated. Parathyroids relatively large. (× 28.)

pret it as an atrophic type, due perhaps largely to the pressure on the gland from adjacent organs. Although these follicles are filled with dense, deeply-staining colloid, it is unlikely that the flattening is due entirely to consequent endofollicular pressure, as follicles are sometimes seen in which the epithelium on the external surface is much more flattened than that on the inner aspect of the follicle. The socalled 'colloid' cells of Langendorff (frequently described by various authors) probably belong to this atrophic type, and have no specific functional significance.

In addition to these peripheral flattened atrophic cells, mention must be made of more advanced types of degeneration, although the latter appear much less frequent in the young rat at 10 weeks than in older animals. These degenerative cells may occur in any part of the gland, either singly or involving an entire follicle (occasionally a regional group of follicles). The degenerating cells may remain in the follicular wall or may be desquamated into the follicular cavity. In rare cases the desquamated epithelium may replace the colloid with an irregular mass of cells in various stages of degeneration.

In the degenerating cells, the cytoplasm loses its typical light granular structure and becomes vacuolated and reticular in appearance, later disintegrating into irregular, usually deeply-staining (eosinophile) masses. The nucleus may be hypochromatic (karyolytic) in type, but more frequently presents various grades of pycnosis (rarely karyorrhexis), especially in the desquamated cells.

As to the frequency with which these degenerative types of cell occur in the thyroid of (apparently) normal rats at 10 weeks, it may be stated that of 9 glands carefully examined in serial sections, one showed rather extensive degeneration, one a well marked area (much less extensive), and four showed traces or small areas in early stages of degeneration. Thus in a majority of the glands, at least slight traces of degeneration could be found, even in normal, apparently perfectly healthy animals.

In older rats (from 3 to 15 months of age), the normal structure of the thyroid gland is essentially similar to that described for the younger rats. The follicles average slightly larger, the maximum sometimes reaching 150 micra, the largest follicles being frequently found at the periphery of the gland. While cubical cells still occur to a variable extent, the average cell is somewhat more flattened than at 10 weeks, especially in the larger peripheral follicles. The colloid is variable in appearance. Occasionally the follicular content may be clear and unstainable. The stroma remains as previously described.

The most striking difference in the thyroid of the older rats is found in increasing prevalence of the degenerative process already described as appearing at 10 weeks. In the older control rats it was found not only more frequently, but more pronounced in character. In extreme cases, the follicles in some regions (see fig. 10) are entirely obliterated, being replaced by masses of epithelial cells with irregularly disintegrated cytoplasm and nuclei in various stages of pycnosis or karyorrhexis. Among 20 glands from apparently normal older rats, only 3 thyroids appeared entirely normal; 6 showed slight degeneration, 5 were moderately involved, while in 6 the degeneration was extensive, involving the larger portion of the gland. The significance of this appearance of degeneration in normal animals will be discussed later.

b. Structure of thyroid gland in young rats held at maintenance

The appearance of the thyroid gland in young rats held at constant body-weight from 3 to 10 weeks of age is shown under low magnification in figures 4, 5 and 6, and more highly magnified in figures 7, 8 and 9. The follicles in general appear in average size smaller than the normal at 10 weeks, although it is somewhat difficult to make exact comparisons, on account of the irregularity in their size. The maximum diameter found in 9 normal glands was about 100 micra, whereas in 14 maintenance rats it was 85–90 micra. The average in both cases was of course much lower, as follicles are found of all sizes down to those with only a minute cavity. Although in the maintenance rats at 10 weeks a few follicles are larger than found in the normal rat at the age of 3 weeks (where the maximum was 70 micra), it is doubtful whether the average follicle is any larger. It is probably slightly smaller.

In size, the epithelial cells of the thyroid follicles in the young rats held at maintenance are variable but distinctly smaller than normal. Even the largest cells found rarely reach the average

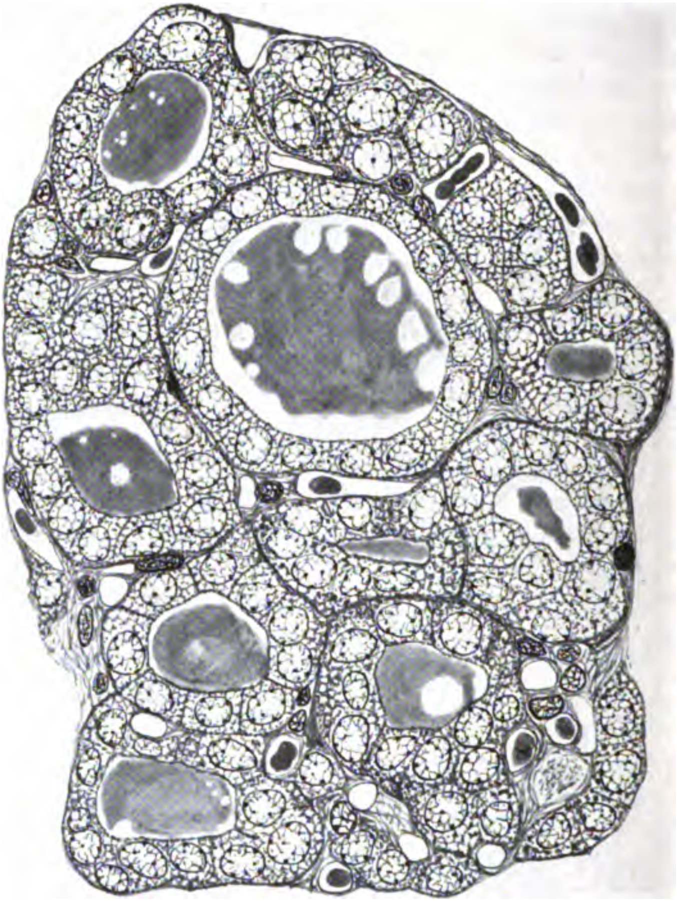

Fig. 7 A portion of the same thyroid gland shown in figure 4 (rat No. 11.63, maintenance from 21 to 72 days of age), magnified to show details of histological structure. The area represented shows the hypochromatic (incipient karyolytic) type of nuclear structure, which is relatively infrequent. Cells reduced in average height (cf. fig. 2). Cytoplasm reduced in amount and vacuolated in structure. Stroma here normal. (× 750.)

normal height (8–10 micra) at corresponding age. The average height in the maintenance rats is about 6 to 7 micra. Since the

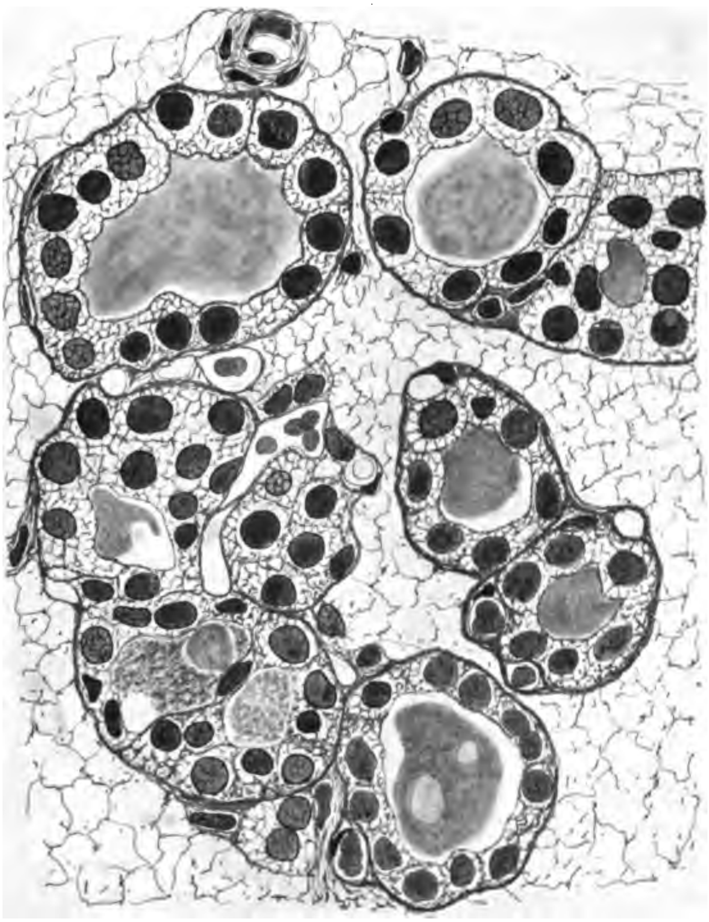

Fig. 8 A portion of the same thyroid gland shown in figure 5 (rat No. S 5.10, maintenance from 21 to 67 days of age), magnified to show details of histological structure. This represents the hyperchromatic (various stages of karyopycnosis) type of structure, which is typical. Follicular cells usually greatly reduced in size. Cytoplasm much reduced in amount and markedly vacuolated ('hydropic degeneration'). Colloid variable in appearance. Interfollicular stroma relatively much increased in volume, due to infiltration of clear ground substance. (× 750.)

whole follicle is but slightly below the normal size, it follows that the cells are more reduced in height than in width. In some glands, the follicular epithelium is very strikingly flattened throughout the entire gland, in no place exceeding 4 or 5 micra in height. Such a condition is never found in the normal gland.

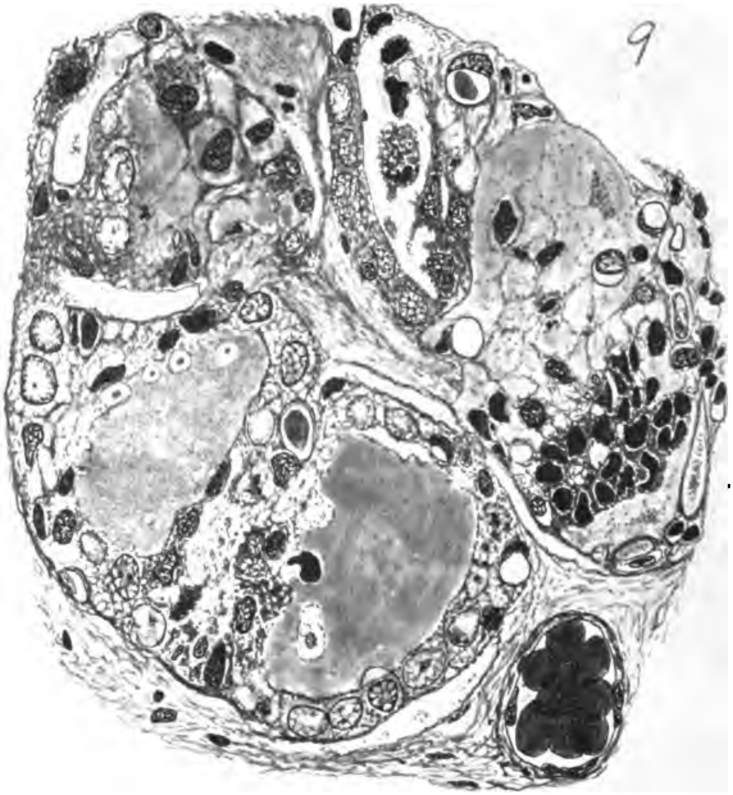

Fig. 9 A portion of the same thyroid gland shown in figure 6 (rat No. 11.64, maintenance from 21 to 73 days of age), magnified to show details of histological structure. This area represents advanced stages of follicular degeneration. Cells in various stages of degeneration and disintegration. Tendency to desquamation with destruction of colloid and obliteration of follicular cavity. Some nuclei appear karyolytic, although karyopycnosis predominates, and karyorrhexis frequently appears. (× 750.)

In the larger peripheral follicles, in which the epithelium is normally flattened, the change is usually not so apparent in the maintenance rats. Even here, however, there is a further flattening of the epithelial cells, which frequently present an endothelial-like appearance, with a height of only 2 or 3 micra.

The cytoplasm of the thyroid epithelium in the maintenance rats has undergone marked changes (figs. 7, 8, 9). In comparison with the normal (fig. 2), it appears that the amount has been greatly reduced, the decrease in the size of the cell being due very largely to loss of cyoplasm.

The cytoplasm is also greatly changed in structure, having apparently undergone a 'hydropic degeneration.' The normal granular appearance of the cells (fig. 2) has been replaced very largely by a distinct reticular appearance (figs. 7 and 8). This appears to be due to a coarse vacuolization of the cytoplasm, a condition rarely found in the normal gland. The vacuolization is especially marked in the few larger cells which have retained more cytoplasm than usual. Occasionally the vacuoles coalesce to form perfectly clear perinuclear areas (see upper part of fig. 8). These clear areas and vacuoles probably represent a watery fluid replacing the normal granular substance, which has been removed and consumed as a result of the inanition.

Where the cytoplasm is very greatly reduced in amount (as in the larger peripheral follicles), the vacuoles may be finer or absent, and the cytoplasm here presents a denser, more deeply-staining (eosinophile), often homogeneous appearance ('colloid' type). In the case of cells in advanced stages of degeneration (which appear much more frequent and more pronounced than in the normal at this age), the cytoplasm is more or less disintegrated and extremely varied in appearance (fig. 9). It usually presents an irregular, deeply-staining (eosinophile), coarsely granular mass, which, especially in the desquamated cells, becomes fragmented and is gradually absorbed, frequently leaving behind the naked, pycnotic nuclei (fig. 9). The cell walls are often distinct in the less modified epithelium, but usually indistinct or lost in the cells markedly degenerated.

The nuclei, generally speaking, appear somewhat more resistant to inanition than does the cytoplasm. In form and size, there appears to be comparatively little change in many of the thyroid nuclei in rats held at maintenance from 3 to 10 weeks of age. The average diameter found in the nucleus of the more nearly cubical cells is between 5 and 6 micra, while the normal at corresponding age is only about 6 micra. It is therefore evident that the nuclear decrease in volume is relatively small. (A decrease of even 10 per cent in the nuclear diameter would correspond to a loss of about 27 per cent in volume, however.) In the more flattened cells (which, as we have seen, usually include the majority), the nucleus is also usually correspondingly flattened, with some loss in volume. Even in the flattened cells, however, the nuclear loss is apparently relatively much less than the cytoplasmic loss in volume.

Although the loss in volume of the nucleus in the thyroid of maintenance rats is relatively small, the structure is usually distinctly modified. Nuclei of the typical normal appearance (as in fig. 2) are rare. The modifications found are varied, but may be classified in two groups—hypochromatic and hyperchromatic.

The hypochromatic type is comparatively rare. In 14 cases of maintenance rats, it appeared extensively but once (shown in fig. 7), and in 3 other cases more or less frequently in scattered cells. The hypochromatic nuclei (fig. 7) usually appear normal in size, or even slightly swollen. They are very light and vesicular in appearance. The nuclear membrane is thin, but sharp and clear; the nuclear network and granules faint and indistinct. The interior of the nucleus is filled with a clear nuclear sap (karyolymph), which is nearly colorless, or with a very pale blue homogeneous tint. In exceptional cases these hypochromatic nuclei apparently undergo complete karyolysis, and disappear without preceding change in form or size.

The hyperchromatic form of nucleus on the other hand, is typical. In the 14 cases of maintenance rats, it appeared more or less extensively in thyroid of all (even in the one case in which the hypochromatic type predominated). Apparently the earliest stage (or at any rate that departing least from the normal type) consists in a mere thickening of the nuclear membrane ("nuclear wall hyperchromatosis") or an increase in the number and size of the nuclear chromatic granules, so that as a whole the nucleus appears darker and more deeply-staining than usual. This form appears normally in the more flattened cells of the larger peripheral follicles, and in the maintenance rats occurs more or less frequently throughout the gland, associated with the shrinkage and flattening of the cells (especially where the nucleus is also flattened).

Associated with or following the nuclear hyperchromatosis just described (in some cases perhaps independently thereof) is found a deepening coloration of the homogeneous nuclear background, which gives the appearance shown in figure 8. This appearance of incipient pycnosis is very characteristic and represents the predominant type of nuclear change observed. Apparently the process involves a progressive dissolution of the basic chromatin masses, which are probably dissolved in the nuclear sap. Various stages occur. The earliest show merely a slight darkening of the homogeneous background, which gradually increases until the nuclear net and karyosomes are obscured and finally disappear (figs. 8 and 9). In size, the nucleus at first shows no marked change (beyond perhaps a slight preliminary shrinkage), but as the process continues the nucleus usually decreases greatly in size. In the typical rounded, homogeneous, dense pycnotic nuclei, the diameter rarely exceeds 4 micra, which represents a tremendous shrinkage. When this stage is reached, the nuclei may apparently persist for a long time without further change, even though the cytoplasm has been reduced to a vestige or entirely removed (as, for example, in the mass of nuclei on the right in figure 9). In many cases of advanced cellular degeneration, however, the pycnotic nuclei become irregular in form and may later undergo karyorrhexis, breaking up into fragments which eventually through karyolysis are dissolved and disappear. Some of these extreme stages of karyorrhexis are shown in figure 9.

In no case was cell-division, either mitotic or amitotic, observed in the thyroid gland of the young rats held at maintenance for several weeks.

The colloid in the thyroid of the maintenance rats is usually nearly normal in appearance (figs. 7, 8), excepting those follicles with advanced degeneration of the epithelium. In these (fig. 9), especially where the epithelial cells are desquamated, the colloid becomes more or less granular and fragmented, finally appearing as irregular masses among the cellular debris or disappearing altogether. The follicles in advanced stages of degeneration are often very irregular in form.

The interfollicular (interstitial) epithelial cells (shown in figures 7 and 8), appear to undergo changes very similar to those in the epithelium of adjacent follicles.

The interstitial connective tissue or stroma of the thyroid in maintenance rats may be nearly normal in amount and structure (as in figure 7), but is frequently increased. This increase is apparently due to an infiltration of clear ground-substance, which in extreme cases gives a very pronounced swollen, 'water-logged' appearance (figs. 5, 8). It is evident that in such a case the weight of the gland might remain normal, even with a marked atrophy of the parenchyma. The fibers adjacent to the follicles may form a denser layer, resembling a basement membrane (fig. 8). The nuclei of the connective tissue cells become shrunken and pycnotic. The vessels may appear nearly normal, although degenerated areas sometimes appear hyperemic.

c. Structure of the thyroid gland in adult rats after acute or chronic inanition

In 6 adult rats whose thyroid was studied the body weight had been reduced 29 to 38 per cent by acute inanition (water but no food) for 8 to 11 days; and in 3 adults the amount of food had been reduced so there was a gradual loss of 33 to 37 per cent in body weight during the chronic inanition period of 5 weeks. The thyroid gland averaged about 0.039 g. (0.24 per cent of the net body weight) in both series, which (compared with controls) indicates little or no loss in absolute weight during inanition. There was found, however, in a larger series of observations an apparent loss of about 22 per cent in the weight of the thyroid gland during chronic inanition (Jackson '15 a).

In the series studied, the structure of the thyroid varied considerably, but on the whole the changes appear to be essentially similar to those already described for the younger rats held at maintenance (really in a condition of chronic inanition).

No constant difference was apparent between the adults with acute and those with chronic inanition. The thyroid follicles are usually but little if any reduced in size. The colloid usually appears nearly normal in amount and structure, and colloid makes up a large portion of the gland. The cells are frequently cuboidal in the central portion of the gland, but on the average somewhat reduced in height. Also, especially in the few cells not reduced in size, the cytoplasm usually assumes a pronounced coarsely vacuolar structure ('hydropic degeneration'), which probably signifies an increase in water with a reduction in the amount of living substance. The nuclei frequently appear but slightly reduced in size, and show a marked tendency to hyperchromatosis (various stages of pycnosis), more rarely hypochromatosis (karyolysis). In the frequent advanced stages of degeneration, desquamation of the follicular epithelium with obliteration of the follicles occurs as previously described in the normal adult gland (fig. 10). Since these degenerative changes occur also in the normal (control) rats, they could not be considered a result of the experiment, except that they usually appear much more extensive and pronounced in the rats subjected to inanition. In the younger rats, however, as we have seen, the degenerative changes occur much less frequently in the normal rats, and the younger animals are therefore more satisfactory for the experiment.

The stroma of the thyroid in adult rats as a rule shows little change during inanition, although there is some tendency to increase in amount, with slight edema (less marked than in the younger rats). There is also frequently a slight hyperemia, especially in degenerating areas. The increase in the volume of the interstitial substance is probably sufficient in most cases to offset the decrease in volume of parenchyma, so that if the colloid remains approximately normal in amount, the stationary absolute weight of the adult thyroid gland during inanition is explained. The greater tendency to loss in weight of the thyroid during chronic inanition and in younger rats is probably due to greater atrophy of the parenchyma, and in some cases to loss of colloid.

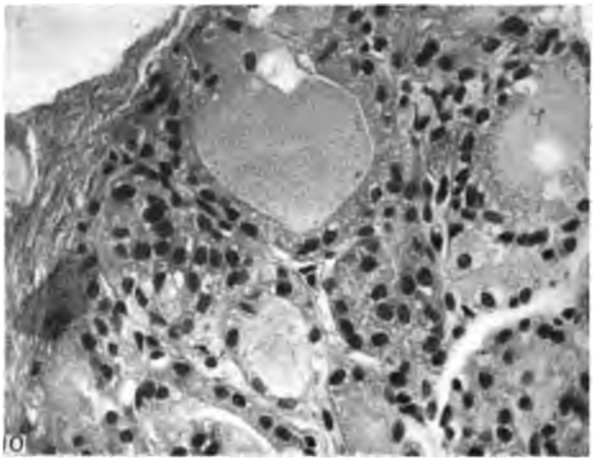
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Fig. 10 From a photograph of a portion of the thyroid gland of a normal adult rat (No. S 14 x, gross body-weight 252 grams), moderately magnified to show typical 'spontaneous' degeneration of the follicles. Nuclei in various stages of pycnosis. Tendency to desquamation of epithelium with obliteration of the follicular cavity. (× 350.)

d. Discussion and conclusions

The question of the extent and significance of the normal variation in the thyroid, with especial reference to the so-called degenerative changes, will be considered first, followed by a discussion of the specific changes produced by inanition.

The extreme variability in the form and structure of the follicular epithelium was observed by many of the earlier investigators. These variations were usually ascribed to age differences or to functional changes. The frequent presence of cells in the follicular cavity was also noted, and the question as to whether the colloid is derived from these desquamated cells or is secreted by the epithelium of the follicular wall was long disputed.

Langendorff ('89), in addition to his well-known 'chief' and 'colloid' cell types, also recognized a retrogressive, degenerative type, which however he did not clearly differentiate from his 'colloid' type (except in theory). Langendorff's results were confirmed by Bozzi ('95), Schmid ('96) and others.

On the other hand, Hürthle ('94) claimed that colloid is formed both by cells of the 'colloid' type and by transformation of desquamated epithelium. Anderson ('94) Müller ('96) and others have opposed the theory that cells of Langendorff's 'colloid' type represent functionally active cells, considering them either functionally exhausted or atrophic, degenerative types, which appears to be the most reasonable interpretation.

De Quervain ('04) made an extensive study of thyroid pathology in man and animals (experiments on dogs and monkeys). From his results, the following conclusions are quoted concerning desquamation and degeneration of the follicular epithelium:

> (p. 139) Wir halten also als Ergebnis dieser 3 Versuchsgruppen fest, dass sich sowohl durch Toxinwirkung, wie auch durch Erzeugung von hochgradigen Zirkulationsstörungen an der Schilddrüse histologische Veränderungen erzeugen lassen, die wesentlich durch Wucherung, Desquamation und Degeneration der Epithelzellen gekennzeichnet sind. . . . . . (p. 142) Wir kommen zu dem Verhalten der Epithelzellen, das uns vor allem interessiert. Die Reaktion derselben auf jeglichen Reiz besteht hauptsächlich in Desquamation. Wenn wir in diesem Abschnitte von Desquamation reden, so verstehen wir darunter die Abstossung von Zellen mit nachfolgendem Ersatz, und nicht etwa nur die postmortale Ablösung der Epithelzellen von der Bläschenwand. Auch die vitale Ablösung der Zellen ohne Ersatz infolge von Nekrose wird uns nur ausnahmsweise beschäftigen. Die Abstossung von Epithelzellen ist an sich ein normaler Vorgang, und lässt sich an völlig gesunden Schilddrüsen nachweisen; doch ist die normale Abstossung eine sehr beschränkte und man wird nur ab und zu in einem Bläschen eine oder einige Zellen bezw. Kerne in Kolloid schwimmen sehen.

Similar retrogressive changes (frequently considered normal) have been described in the thyroid gland in children, and even in newborn and late fetuses (Zielinska '94, Elkes '03, Hesselberg '10, Isenschmid '10, Gleim '12).

Watson ('09) in 40 wild rats (species not stated) weighing 60 to 370 grams found wide variations in the thyroid structure, which he described under four types. His first type presents large colloid-filled follicles with flattened cells and darkly-stained nuclei. His second type shows smaller follicles with small cuboidal cells with relatively little cytoplasm and with rounded nuclei (appearing deeply-stained in his figure 2 A). In type 3, the follicles are small, cells large with abundant cytoplasm, and colloid faintly-staining. In type 4, the follicle cells are detached and distorted, with pycnotic nuclei and cytoplasm of variable amount and appearance. Types 1 and 2 (with intermediate forms) predominated in 31 of the 40 glands, type 3 in 5 and type 4 in 4 cases.

Watson concluded that these variations found in thyroid structure "have been induced by dietetic or other factors in the animal's environment." He thought that they did not represent different stages of functional glandular activity (related to digestion), since they were not correlated with the nature of the stomach contents. He does not mention the possibility of inanition, which according to my results might be the cause of his types 1 and 2. The fact that type 1 was found in animals with both full and empty stomachs does not disprove this, as the history previous to capture is unknown and the length of time the animals were fed is not stated. Type 3 is obviously that usually described as normal and type 4 corresponds to the degenerative type which I have found frequently in apparently normal animals.

In previous work, Watson ('05) had studied the effect of a meat diet upon the thyroid gland of 12 tame rats (11 were 6 to 12 weeks of age, 1 adult) with 8 controls. The experiment lasted 6 weeks to 4 months; and the meat-fed animals were usually retarded in body weight. Ten of the 12 showed marked changes in the thyroid, including congestion, epithelial proliferation of the follicles and degeneration of the colloid. (Similar results in meat-fed rats were obtained by Tanberg, cited by Biedl '13.)

In a later paper, Watson ('07 a) studied the thyroid in 20 wild rats, 10 of which were killed immediately after capture and 10 fed bread and milk for 10 weeks. The former showed large colloid-filled follicles with flattened epithelium; the latter had smaller follicles, with little colloid and cubical epithelium. Waters ascribes the change to the difference in diet; but here, as also in his meat-fed rats, it is difficult to exclude the factor of inanition (inadequate nutrition). He also failed to note that the degeneration may occur apparently spontaneously. In another paper Watson ('07 b) finds in young rats fed on oatmeal diet for several weeks a marked enlargement of the thyroid (0.078 per cent of the body weight as compared with 0.029 per cent in controls). In some cases, the thyroid epithelial cells were swollen and detached.

Rebello and DaCosta ('10) describe the thyroid of the normal rabbit as variable in structure with the occurrence of epithelial desquamation and degeneration, resulting in obliteration of the follicles in a manner very similar to that described above for the rat. They consider this a normal physiological process following functional activity. However they find the degeneration markedly increased by the injection of thyroid-proteid extracts. They also observed similar degenerated areas in the thyroid of a normal young dog.

Douglas ('15) in an extensive study of the effects of various diets upon the thyroid of pigeons, chicks and rats, described four types of structure, somewhat similar to those of Watson. He concludes that:

> The histological appearances do not represent different stages of secretion, comparable to those of secreting glands engaged in the process of digestion. Under similar conditions and in animals fed on similar diets, the appearances in the thyroid differ very markedly. One observes all stages from the type with large vesicles, full of colloid with flattened cells, to that of a thyroid with no colloid and columnar shaped cells. Also the thyroid may be wholly or partly disintegrated. The variation in appearance of the thyroid seems to depend to some extent on the nutrition, and is thus only in this way dependent on the diet.

Out of 31 rats (species not stated) with "ordinary laboratory diet of bread" Douglas found the degenerative type of thyroid only once, though it occurred frequently in pigeons and chicks. It occurred very frequently (8 of 11 cases), however, among rats fed various food mixtures with fat. The normal rats usually presented thyroid follicles with cubical cells and a moderate amount of colloid.

The variability in the structure of the thyroid gland and its physiological and pathological relations have been discussed by Marine and Lenhart ('09 a, '09 b, '11 a, '11b, '11 c) in a series of papers. On the basis of an extensive investigation of the human and animal (dog, sheep, ox, pig, etc.) thyroid, they distinguish as 'physiological' the following: (1) normal resting gland, with low cuboidal epithelium; (2) hyperactive stage, with hyperemia, cells becoming hyperplastic and columnar, colloid disappearing; (3) colloidal stage (of recovery), in which conditions again return to (1) with abundant colloid. If hyperactivity continues without rest, however, colloid disappears and the cells die of exhaustion, becoming progressively degenerated and desquamated with varied degrees of disintegration. The nuclei in this case are described as enlarged, often hyperchromatic, and quite variable in appearance. Similar desquamation and degeneration of the follicular epithelium is described in normal senile atrophy. The flattened type of epithelium, indicating a minimum functional activity, may be experimentally produced by administration of iodine in the diet; whereas a diet without iodine induces the columnar, hyperactive type of thyroid cells.

The variability in structure due to the extremely sensitive nature of the thyroid gland is emphasized by Marine and Lenhart ('11 c) as follows:

> It has long been recognized and frequently emphasized by us that the thyroid tissue is extremely labile—reacting quickly to relatively slight physiological variations in the body metabolism, and for this reason may show even daily histological changes within narrow limits.

Marine ('15) also notes that thyroid glands undergo autolysis in a few hours after removal from the body, especially if kept near the body temperature, resulting in desquamation of the alveolar epithelium.

In a recent communication in reply to a letter of inquiry by the author, Dr. Marine states that he has frequently observed the so-called degenerative changes in the thyroid of the rat and other animals, and comments as follows:

> I personally consider this change as a kind of autolysis. Autolysis in the thyroid is perhaps more easily recognized than in any other organ of the body because of its simple architecture. The change of which you speak consists of a desquamation of the alveolar cells and a breaking up of the cell membrane, giving a ragged appearance to the free border of the cells. This is always more marked in the slightly hyperplastic stages. Much has been made of this by various experimenters in an attempt to bring it into relation with the specific experiments that they were studying. It is of too general occurrence to have so varied a significance. The rat and the chick we have found to show these changes most frequently. One sees it in all chicks or rats that are autopsied some hours after death and even in rats sacrificed and autopsied at once, as all ours have been just for the purpose of eliminating this autolysis. I also think that formalin fixation is favorable for its development, as one rarely sees it in strictly fresh thyroids that have been fixed with metallic salts. In human thyroid pathology, as you are aware, this change has been described in almost every paper and in connection with the Basedow syndrome. Most writers who have studied the human thyroid have attempted to ascribe some peculiar toxic value to it. This is wrong. It is present in the cretin thyroid or in any active hyperplastic thyroid as well. . . . . . In conclusion, therefore, I believe that this change is quite common in rats and fowl thyroids generally, and is of frequent occurrence in all thyroids unless the special precaution is taken of obtaining the tissues strictly fresh and fixing them in solutions other than formalin. I do not think it is any more common in starvation experiments than in other groups.

It might also be added that glands which have not been placed promptly in the fixative, and in which the process of post mortem autolysis has begun, sometimes show considerable differences in appearance at different depths from the surface. The fixation is better in the superficial layers than in the deeper strata which require a longer time for penetration.

In order to compare the structure of the thyroid gland, and especially the occurrence of degeneration, in the rats used by me with those elsewhere, I have obtained material from various localities. Professor Addison, of the University of Pennsylvania, kindly furnished mounted specimens of the thyroid from 3 normal albino rats. In a young rat (121 days), the structure was found nearly normal, the epithelial nuclei rarely showing incipient pycnosis. In 2 older rats, however, (200 and 452 days), there was found a considerable amount of epithelium showing desquamation and typical degenerative changes. In the thyroid of a gray rat (said to be a hybrid gray-albino) sent by Professor Evans, of the University of California, the structure was found nearly normal, but a few areas showed typical degeneration with partial desquamation of the epithelium.

Through the courtesy of Professor Bensley and Mr. Burgett, I obtained mounted specimens from 8 thyroids of normal albino rats (body weight 117 g. to 287 g.) from the University of Chicago laboratories. Of the 8 glands, 2 showed extensive follicular degeneration, 1 moderately extensive and 2 very small areas of slight degeneration. Only 3 appeared perfectly normal throughout.

Concerning the degenerative appearances found in the thyroid gland, Dr. Bensley writes:

> There are two circumstances which should be borne in mind in connection with these changes in the thyroid gland, which frequently, I think, are responsible for similar changes in tissues which were normal to start with, namely: drying of the surface of the gland when adequate care is not taken in transferring to weighing bottles; and pinching with forceps in the process of extraction. The latter frequently initiates a rapid change in the cell which is marked by acid reaction to indicators, and by greater affinity for basic stains in the fixed preparation, with loss of characteristic structure by the nucleus.

In connection with the suggestion by Dr. Bensley of the importance of traumatic injury during the removal of the organ in producing degenerative changes, the observations of Sutherland ('15) upon pycnotic changes in nuclei of the spinal cord near the seat of injury may be cited.

From the foregoing, it appears that the characteristic follicular degeneration of the thyroid appears to a variable extent in apparently normal rats, both wild and albino, in widely separated localities.

Summing up the literature cited, together with my own observations, we may conclude that the thyroid gland shows an unusual amount of normal variability in histological structure, doubtless due at least in part to functional stages which are as yet imperfectly understood. Furthermore, on account of the extreme sensitiveness of the thyroid, it reacts strongly to various influences by changes which lie on the border-line between normal and pathological. So-called degenerative conditions (desquamation and degeneration of epithelium) appear to a limited extent even in the thyroids of normal animals (including man), although these conditions probably have no physiological significance. Under various abnormal conditions, these retrogressive changes appear to be increased in extent and intensity.

Having reviewed the variable structure of the thyroid, we may now consider briefly the specific effects of inanition, as found by the few investigators who have studied the histological changes.

Barbèra ('02) studied the effects of acute inanition upon the thyroid gland in 3 rabbits and 1 dog, with controls of the same sex and from the same mother. The rabbits were starved 7 to 11 days, and the dog 21 days, with loss of about 30 per cent in body weight in each case. No data are given as to weight of the gland. No measurements of the entire cells are stated, but they were found reduced in size, the loss in the cytoplasm being greater than in the nucleus. The average of a large number of measurements of the nuclear diameters showed in the rabbit 5.73 × 4.99 micra in the controls, and 5.75 × 3.84 in the starved aninals. This would indicate a reduction in size with relative elongation of the nuclei. This elongation of the nucleus was not found in the dog, however, where the average of 5.11 × 4.62 micra in the controls was reduced to 4.44 × 4.28 micra in the starved. The intercellular substance was also found reduced in amount, but colloid formation appeared to continue normally (hence no symptoms of hypothyroidism).

As above stated, I have likewise found in the rat thyroid during inanition a slight reduction in the size of the nucleus, which is usually much less marked than the decrease in cytoplasm. I find the shape of the nucleus dependent upon the form of the cell; in cases where the cell becomes flattened, the nucleus becomes correspondingly elongated, but otherwise it usually retains its spherical or ovoidal (ellipsoidal) form. Morgulis ('11) found a tendency to relative elongation in the nuclei of various organs in Diemyctylus during starvation, but not in the liver and pancreas of the albino rat.

Traina ('04) finds in the thyroid of the rabbit during inanition a decrease of about 30 per cent in the volume of the cells, the loss being relatively greater in the cytoplasm than in the nucleus. The cells lose more in height than in breadth; the nucleus remains distinct and regular in outline. The fatty granules (previously described in the thyroid by Erdheim '03) remain in the atrophic cells unchanged in position, number, form and size.

Missiroli ('11) in rabbits finds the thyroid structure correlated with the stage of digestion present. When food is withheld, the colloid is no longer eliminated but accumulates and distends the follicles. In some cases of prolonged fasting, the colloid may undergo 'fatty degeneration.' In advanced stages of inanition, nuclei and protoplasm undergo atrophy, and the interstitial (connective) tissue appears increased. On refeeding, the colloid accumulated in the follicles is rapidly eliminated.

Mrs. Thompson ('11) states that:

> In a dog which had been subjected to a few days' inanition, the thyroid (hardened in strong Flemming's fluid) appears very different from the normal. The cells appear swollen rather than proliferated, and in some cases vesicles are filled with cells. The vesicles are shrunk so as to assume various shapes, and much intervesicular material appears. The general appearance is as if the gland had been roughly squeezed, so that the vesicles are any shape but spherical. The change wrought by inanition seems to make the structure of the gland tend towards that of the parathyroid.

It is doubtful to what extent the changes described by Mrs. Thompson were actually due to inanition, since the inanition period was relatively short (for a dog) and the normal variation in structure is uncertain. She describes a similar appearance in the thyroid of a cereal-fed dog, while that of a meat-fed dog was normal.

In general, therefore, we find that the observations upon the structure of the thyroid during inanition, including those of previous observers and my own, while differing in various minor details, agree in the main. The epithelium of the thyroid follicles apparently undergoes at first a simple atrophy, which affects the cytoplasm more than the nucleus. The cells thus become reduced in height, with relatively large nuclei. In advanced stages of inanition, degenerative phenomena (found also to a limited extent even in the normal gland) become increasingly evident. The follicular cells may remain in situ, but usually are desquamated, replacing the colloid (which otherwise remains nearly normal). The cytoplasm, typically vacuolated in the earlier stages, may become collapsed, deeply-staining and more or less homogeneous ('colloid' type) in appearance, or may disintegrate, forming an irregular granular mass. The nucleus usually becomes hyperchromatic, undergoing successive stages of karyopycnosis, ending in extreme cases in karyorrhexis. Sometimes, however, it becomes hypochromatic, and undergoes karyolytic changes.

It is somewhat remarkable that these characteristic changes during inanition,—cell-atrophy with characteristic, more or less extensive nuclear and cytoplasmic degeneration,—are likewise found to a greater or less extent associated with a wide range of other conditions. In the foregoing pages, changes in thyroid structure somewhat similar to those associated with the various degrees of inanition have been mentioned in connection with the following:

1. Spontaneous (?) degeneration (in normal animals without apparent cause).
2. Age changes (tendency of cells to become flattened with age; senile atrophic changes).
3. Functional changes (varied changes due to functional activity; atrophy in the exhaustion following hyperactivity).
4. Changes due to effects of diet (meat and other special diets; administration of iodine).
5. Toxic effects (changes produced by injection of various toxic substances, proteid extracts, etc.).
6. Circulatory disturbances (stasis produced by ligation of vessels, etc.).

7. Pathological conditions (thyroiditis, exophthalmic goiter, etc.), probably due to infections.

8. Results of imperfect fixation.

9. Post mortem changes (due to mechanical injury, post mortem autolysis, etc.).

To this should be added the statement that very similar cell-changes have been described, especially by pathologists, in various tissues of the body, most frequently in the epithelia of the various parenchymatous glands. Changes in the renal epithelium following ligation of vessels, infarcts, intoxications, infections, post mortem changes, etc., form a familiar example, in which the phenomena are in many respects strikingly similar to the degenerative changes above described for the thyroid gland.

The remarkable similarity in the degenerative changes thus found in various organs and under apparently widely different conditions naturally raises the question as to whether the underlying fundamental process may not be at least in some respects essentially similar in all cases. It may be possible, for example, that cellular inanition may be produced in different ways, and may therefore be the essential factor in the process of degeneraation arising from varied causes. If, as is now generally assumed, cellular metabolism is accomplished by means of numerous varieties of intracellular enzymes, one could easily understand how anything which would destroy or interfere with the activity of any of the enzymes necessary to anabolism might thereby produce a condition of cellular inanition, even in the presence of an abundant food-supply. The probable importance of autolytic enzymes in the phenomena of cell-degeneration, especially in the nuclear changes of pycnosis, karyolysis, etc., has been emphasized by Wells ('14) and others. A final solution of the problem is impossible at the present time, on account of our scanty knowledge of normal cell-physiology; but it seems probable that many of the phenomena of cellular degeneration from various apparent causes will ultimately be explainable as due essentially to cell-inanition.

THE PARATHYROID GLAND

The normal structure of the parathyroid glands will be considered first, followed by a discussion of the changes produced during inanition.

a. Normal structure of the parathyroid glands

As is well known, there is in the rat a single pair of parathyroid glands, said to correspond to parathyroid III. In one exceptional case, I found a parathyroid (see table 1) on one side only. I have never observed the accessory parathyroids described by Erdheim (cited by Biedl '13). The parathyroids are located upon the external surface of each lateral lobe of the thyroid gland, usually somewhat posteriorly, though occasionally more anteriorly (figs. 1, 3, 4, 5, 6).

Ochsner and Thompson ('10) state that the parathyroids of the rat are located within the thyroid lobes near the upper pole. I find, however, that there is considerable variation; they occur slightly more frequently opposite the middle third of the thyroid lobe, but often at more cephalic or caudal levels. Of 59 glands whose position was noted, 18 were located in the cephalic third of the thyroid, 20 in the middle third, 13 in the caudal third, 6 at the junction of the cephalic and middle thirds, and 2 at the junction of middle and caudal thirds. The parathyroids of the two sides are usually, but not always, at nearly the same level. In 3 cases observed, one parathyroid was located in the cephalic third of the lateral lobe of the thyroid, while the opposite parathyroid corresponded to the caudal third of the thyroid.

The parathyroid gland lies partly embedded within the thyroid, about one-fourth to one-third of its surface usually forming an external free or exposed surface (figs. 1, 3, 4, 5, 6). Sometimes it may project more extensively from the thyroid surface; in other cases it lies more deeply, rarely entirely embedded within the thyroid.

As to size, the dimensions of the parathyroid gland at various ages are given in the following table.

TABLE I

Dimensions of parathyroid glands in albino rats. Measurements from mounted sections

RAT NO. AND SEX	AGE	BODY-LENGTH	BODY-WEIGHT GROSS (AND NET)	DIMENSIONS OF PARATHYROIDS		AVER. DIAM.
				a	b	
	days	*mm.*	*grams*	*mm.*	*mm.*	*mm.*
A. Normal (control) rats						
St 32.1 f	½	46	5.0(—)	0.22×0.18×0.26	0.26×0.18×0.26	0.23
V 2.1 m	14	89	22.7(—)	0.34×0.18×0.30	0.34×0.18×0.36	0.28
S 9.47 f	22	95	25.5(23.3)	0.33×0.32×0.38	0.28×0.38×0.38	0.35
St 32.2 m	21		27.2(—)	0.46×0.32×0.48	0.53×0.36×0.50	0.44
St 5.1 f	56		63.0(—)	0.62×0.56×0.62	0.56×0.50×0.52	0.56
St 12.54 f	110	166	121 (116)	0.96×0.58×0.72	0.83×0.64×0.72	0.76
S 5.4 f	74	168	126 (118)	0.64×0.50×0.40	0.54×0.50×0.36	0.49
St 10.24 f	117	169	135 (129)	1.21×0.65×0.90	0.94×0.70×0.90	0.88
H 24.3 f	232	188	143 (138)	0.66×0.56×0.88	0.50×0.56×0.72	0.64
H 65.7 f	98	179	144 (139)	0.57×0.43×0.64	0.55×0.40×0.80	0.57
H 60.3 f	78	182	146 (138)	0.47×0.58×0.74	0.75×0.48×0.74	0.63
H 68.3 f	74	185	158 (151)	0.45×0.48×0.58	0.46×0.48×0.52	0.50
H 42.3 f	182	195	170 (160)	0.55×0.64×0.74	0.78×0.60×0.70	0.67
S 5.3 m	74	180	172 (167)	0.62×0.36×0.47	(not observed)	0.48
H 47.3 m	145	201	174 (168)	0.54×0.64×0.72	0.66×0.54×0.80	0.65
H 27.3 f	253	195	175 (166)	0.84×0.58×0.90	0.89×0.60×0.80	0.77
MI 2 m	74	208	181 (173)	0.49×0.58×0.74	0.56×0.46×0.67	0.58
H 34.3 f	224	194	181 (173)	0.70×0.54×1.12	(1 gland absent)	0.79
H 34.6 f	225	205	194 (188)	0.57×0.70×0.86	0.84×0.56×0.76	0.71
S 33.116 f	340	195	194 (188)	0.79×0.60×0.86	0.90×0.68×0.70	0.76
H 70.3 m	70	194	208 (198)	0.63×0.48×0.80	0.79×0.53×0.80	0.67
H 27.6 m	254	214	229 (222)	0.75×0.75×0.85	0.88×0.60×0.80	0.77
H 50.3 m	141	211	230 (222)	0.75×0.60×0.90	0.77×0.72×0.90	0.77
S 14 x m	?	205	252 (247)	0.68×0.55×0.65	0.72×0.55×0.80	0.66
H 54.3 m	101	222	284 (275)	0.86×0.60×0.80	0.66×0.64×0.80	0.73
St 14 m	442	239	322 (314)	0.88×0.72×0.96	1.07×0.85×0.90	0.90
B. Rats at maintenance from 3 to 10 weeks of age						
S 5.10 f	67	100	22.7(21.0)	0.49×0.40×0.42	0.52×0.37×0.50	0.45
S 11.63 f	72	95	23.8(22.6)	0.50×0.50×0.50	(not observed)	0.50
S 11.65 m	73	100	23.8(22.5)	0.48×0.44×0.53	0.47×0.48×0.52	0.49
S 11.64 f	73	100	24.2(22.9)	0.61×0.55×0.65	0.68×0.50×0.60	0.60
S 12.69 f	66	100	24.5(22.7)	0.43×0.50×0.65	0.46×0.55×0.55	0.52
S 6.23 f	72	102	26.1(24.5)	0.72×0.40×0.30	(not observed)	0.47
S 5.8 f	73	105	27.6(24.6)	0.50×0.45×0.35	0.50×0.45×0.35	0.43

TABLE 1—Continued

RAT NO. AND SEX	AGE	BODY-LENGTH	BODY-WEIGHT GROSS (AND NET)	DIMENSIONS OF PARATHYROIDS		AVER. DIAM.
				a	b	
	days	*mm*	*grams*	*mm.*	*mm.*	*mm.*
			C. Adult rats with acute inanition[1]			
M 1 m	(?)	205	248 to 168(165)	0.50×0.60×0.80	0.45×0.64×0.80	0.63
M 2 m	(?)	(?)	254 to 170(167)	0.69×0.48×0.80	0.57×0.52×0.80	0.64
S 16 f	(?)	195	267 to 190(186)	0.75×0.51×0.52	0.78×0.50×0.55	0.60
S 27 m	(?)	215	320 to 223(219)	0.77×0.60×0.80	0.85×0.60×0.80	0.74
S 25 m	(?)	205	328 to 202(198)	0.73×0.52×0.70	0.88×0.58×0.66	0.68
			D. Adult rats with chronic inanition[1]			
M 3 m	(?)	175	188 to 125(122)	0.48×0.48×0.96	0.49×0.66×0.72	0.63
M 6 m	(?)	175	220 to 138(134)	0.35×0.45×0.70	0.35×0.70×0.75	0.55
M 11 m	(?)	190	264 to 163(158)	0.55×0.52×0.72	0.52×0.50×0.70	0.59

[1] Both initial and final body weights given.

In the first column, the letters 'St', 'V,' etc., refer to different series of rats used. The number preceding the decimal point is the litter number; the number following is the individual number. 'm' refers to male, 'f' to female. Under 'Dimensions of parathyroids,' the measurements for the two glands are listed separately in columns 'a' and 'b'.

The cephalo-caudal length (which is always the first of the three dimensions given in the table) was calculated from the number of sections, of known thickness, through which the gland extended. The other dimensions represent the largest and smallest diameters measured in the largest cross-section of the gland, in the mounted sections. It should be noted, therefore, that these dimensions are smaller than in the fresh gland, as each linear dimension has probably undergone a shrinkage of at least 10 per cent in the process of fixation, dehydration and embedding.

Generally speaking, the parathyroid presents a slightly flattened, ellipsoidal form, the relative dimensions usually corresponding in a general way to those of the associated lobe of the thyroid. That is, in cross-section the outline usually appears somewhat compressed medio-laterally; but the cephalo-caudal dimension is greatest in less than half of the cases. It will be noted, however, that the parathyroid is frequently nearly spherical in form, the three axes being approximately equal (table 1; figs. 1 and 3 to 6).

The normal growth in the dimensions of the parathyroid is evident from the table. In the pair of newborn parathyroids, the average diameter is 0.23 mm.; at 2 weeks it is 0.28 mm.; at 3 weeks 0.35 to 0.44 mm.; and at 8 weeks, 0.56 mm. In 5 rats from 70 to 74 days of age, the average is 0.54 mm. (range 0.48 to 0.67 mm.). The dimensions increase very irregularly up to 0.90 mm. average in a rat of 322 g. gross body weight, 442 days of age.

In spite of the comparatively small number of observations and the admitted inadequacy of the average diameter as a measure of the size of the parathyroid, it is believed that these data are useful for purposes of general comparison. The data for the individual parathyroids were compared with the weights of the corresponding thyroids (not shown in the table), but there is apparently no close correlation between them.

A point of special interest, which is not apparent from casual inspection of the table, appears more clearly when the observations are plotted graphically according to the body weight and average diameter of the parathyroid. The gland appears to be relatively larger in the females. Further observations will of course be necessary to establish this point with certainty; but if true, this will place the parathyroid in a class with the hypophysis and suprarenals, in which the female rats possess relatively larger glands.

The normal structure of the parathyroid gland in the albino rat at 3 weeks of age is shown in figure 11. The epithelial cells are grouped in irregular, closely-packed masses, occasionally showing a cylindrical or cord-like arrangement.

In size the parathyroid epithelial cells vary considerably, the average being about 6 x 8 micra, or slightly larger. They are thus considerably smaller than the thyroid epithelium of a corresponding gland. The cell boundaries are usually distinct.

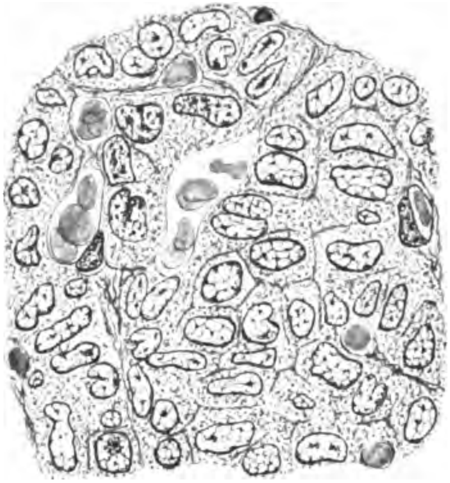
The cytoplasm is relatively small in amount, forming a narrow zone 1 to 2 micra in width around the nucleus. The cytoplasm is usually finely granular and pale reddish-violet in appearance (with Zenker fixation and haematoxylin-eosin stain). No 'oxyphile' (eosinophile) cells are present.

The nuclei of the parathyroid epithelium (fig. 11) vary considerably. They usually appear elongated, the smaller rounded forms perhaps representing cross-sections of ellipsoidal nuclei. There is considerable variation in size (average about 4 x 6 micra) and form. The nuclear outline frequently presents a notched or lobulated appearance.

In structure, the parathyroid nuclei are similar to those of the thyroid, presenting a distinct, deeply-staining nuclear membrane, and a faint nuclear network with usually one or two distinct nucleoli (karyosomes), in a clear, light, homogeneous background corresponding to the nuclear sap (karyolymph). Mitoses are relatively abundant at this stage, two or three being found in each cross-section near the center of the gland.

The parathyroid gland, like the thyroid, is in general fairly uniform throughout in structure at this time, but certain irregularities may appear, even at this age. In the most superficial stratum of the gland, especially on the external free surface (not in contact with thyroid) modified cells may appear. These cells usually form small groups of somewhat atrophic appearance. The cytoplasm is more irregular, vacuolated or denser and deeply-staining. The nuclei of these cells are generally somewhat smaller than usual, often flattened, and with more or less deeply-staining homogeneous appearance characteristic of the earlier stages of pycnosis. Such atrophic cells rarely occur except near the surface, and perhaps represent a mild form of pressure-atrophy (as found also near the surface in the thyroid).

The interstitial connective tissue forms a delicate, fibrous stroma, like that of the thyroid, except that it is usually not so highly vascular (fig. 11). In a few places, it is somewhat more abundant, forming scattered, irregular, light streaks observed in the interior of stained cross-sections. These streaks apparently become more distinct in the older rats.



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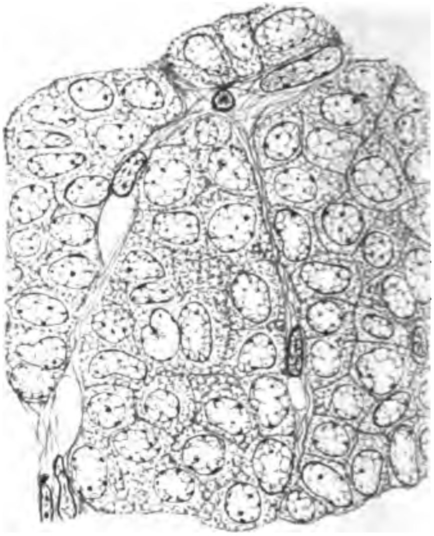

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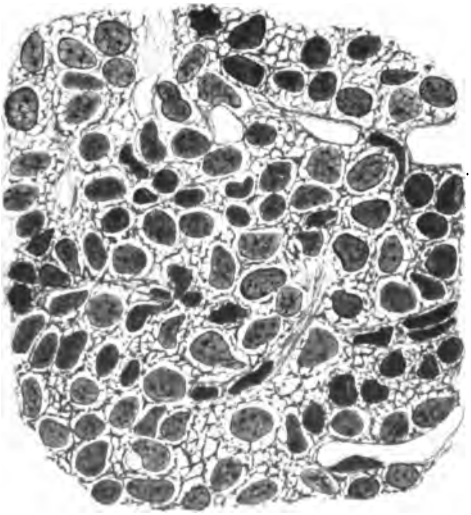

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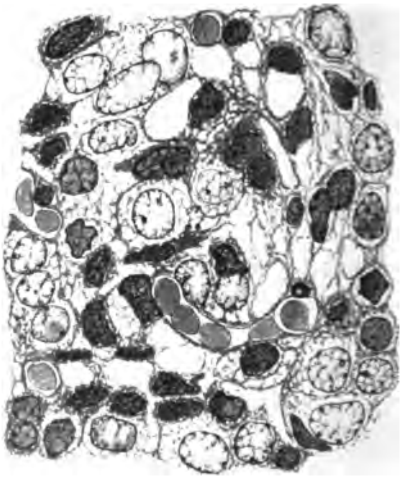

14

In the parathyroid at 10 weeks, the structure is essentially the same as at 3 weeks (fig. 11), no important change in the appearance or average size of the cells being observed. Mitoses appear somewhat less frequently, only 1 or 2 in a section. The atrophic or degenerated types appear usually somewhat more frequently and more pronounced in character, however. They most commonly appear in the superficial stratum, especially at the exposed surface (not in contact with thyroid); but occasionally in the interior also, usually in scattered areas. The nuclei of these cells are usually deeply-staining and pycnotic, though vesicular karyolytic forms also occur. The cytoplasm in these atrophic types is usually rarefied and vacuolated in appearance, but occasionally somewhat coarsely granular and deeply-staining (eosinophile), suggesting in some respects the 'oxyphile' type described as normal by some authors for the

Fig. 11 A small portion of the parathyroid gland shown in figure 1 (rat S 9.47, normal 3 weeks), magnified to represent the details of the normal histological structure (which is essentially the same as at 10 weeks). Epithelial cells in irregular masses; cytoplasm granular, scanty in amount; nuclei irregularly elongated in appearance. Stroma scanty, vascular. (× 750.)

Fig. 12 A small portion of the parathyroid gland shown in figure 4 (rat No. S 11.63, held at maintenance from 21 to 72 days of age), magnified to show details of histological structure. This area represents the hypochromatic (incipient karyolytic) type, which is relatively infrequent. The nuclei appear light and swollen. The cytoplasm here is apparently not reduced in amount, but shows tendency to vacuolization and disappearance of the normal granulation. Stroma normal. (× 750.)

Fig. 13 A small port on of the parathyroid gland shown in figure 5 (rat No. S 5.10, held at maintenance from 21 to 67 days of age), magnified to show the details of histological structure. This area represents the typical hyperchromatic type (various stages of karyopycnosis). Cytoplasm more or less reduced in amount, with marked vacuolization ('hydropic degeneration'). Stroma normal. (× 750.)

Fig. 14 A small portion of the parathyroid gland shown in figure 6 (rat No. S 11.64, held at maintenance from 21 to 73 days of age), magnified to show the details of histological structure. This area represents a somewhat advanced stage of degeneration, which frequently involves small, scattered masses, but is rarely extensive. The nuclei are partly of the hypochromatic (karyolytic), and partly of the hyperchromatic (karyopycnotic) type. Around the former, the cytoplasm is usually vacuolated ('hydropic degeneration'); while around the latter type cytoplasm appears reduced in amount, deeply-staining (eosinophile) and frequently coarsely granular in appearance. (× 750.)

parathyroid of various animals (including man). Sometimes spaces are seen between the larger bundles of connective tissue and the adjacent parenchyma. These spaces somewhat resemble in appearance the paratrabecular and subcapsular lymph-sinuses in lymph-nodes.

In the older and adult animals, the parathyroid gland shows essentially the same structure as described at 10 weeks. The cells average perhaps slightly larger, the nuclei being typically elliptical and averaging between 4 x 6 and 5 x 7 micra. The cytoplasm forms a light granular zone 1 to 2 micra in width. Mitoses are not found in the adult rats. The atrophic or degenerative types of cells occur frequently, though usually in limited, scattered areas, especially near the exposed surface of the parathyroid.

b. Structure of the parathyroid glands in young rats held at maintenance

If in the rats held at maintenance (constant body-weight) from the age of 3 to 10 weeks the dimensions of the parathyroid glands be compared with those of the controls at 3 and 10 weeks (table 1), it appears that the parathyroid has not shrunken in size during the experiment. On the contrary it has even apparently increased so that the dimensions are nearly as large as those of the full-fed controls of the same age but several times larger in body-weight. As shown in table 1, the average diameter of the normal parathyroid at 3 weeks is about 0.40 mm., at 10 weeks, 0.54 mm.; in maintenance 3 to 10 weeks, 0.49 mm. Thus the parathyroid in young rats held at maintenance becomes relatively very large in comparison with the thyroid (which usually decreases somewhat), as is apparent upon comparison of the parathyroids in figures 1 and 3 with those in figures 4, 5 and 6.

The changes in the histological structure of the parathyroid gland during inanition are on the whole less marked than in the thyroid gland, although in most respects similar in character. In the parathyroids of rats held at maintenance from the age of

3 to 10 weeks, the epithelial cells show a variety of changes in structure, but it is doubtful whether there is in most cases any marked decrease in the average volume of the cells.

The cytoplasm, however, has undergone structural changes similar to those described for the thyroid cells (though somewhat less marked), its finely granular structure usually showing more or less marked vacuolization or 'hydropic degeneration' (figs. 12, 13, 14). In some cases, however, especially with advanced karyopycnosis, the cytoplasm may appear somewhat diminished in amount, and with deeply-staining (eosinophile), coarsely granular structure. The cell-boundaries sometimes remain distinct (fig. 12), but are frequently indistinct (figs. 13 and 14).

Two distinct types of nuclear change occur (similar to those found in the thyroid). In the first or hypochromatic type (fig. 12) the nuclei appear vesicular, swollen and very lightly stained. This type is relatively less frequent (as in the thyroid) and may appear in all the cells over considerable areas (as in fig. 12), or in scattered cells or small groups (as in fig. 14). This type of nucleus probably represents an early stage of karyolysis, though it is doubtful whether any nuclei actually reach dissolution in the specimens studied.

As in the thyroid, the more frequent type of nuclear change in the parathyroid in young rats held at maintenance is in the direction of hyperchromatosis, representing various stages of karyopycnosis (figs. 13 and 14). For the most part, however, the parathyroid nuclei do not go beyond the earlier stages, merely showing a homogeneous background more deeply colored so as to obscure the internal nuclear structure. In these nuclei, there is little if any decrease in size, and this type frequently occurs throughout practically the entire gland. Less frequently more advanced stages of pycnosis are found, in which the nuclei are diminished in size, with homogeneous deep stain (fig. 13). The hyperchromatic and hypochromatic types of cell-degeneration may sometimes occur intermingled, as in figure 14.

No advanced stages involving karyorrhexis were found. Neither mitosis nor amitosis was observed in any case.

The interstitial tissue or stroma of the parathyroid rarely shows any noteworthy changes. In some cases it appears slightly increased in amount, with edemic appearance, but not so often as in the thyroid.

c. Structure of parathyroid in adult rats after acute and chronic inanition

In adult rats subjected to acute or chronic inanition, the dimensions of the parathyroid gland are given in table 1. While it is impossible to draw any final conclusion, on account of individual variation and the small number of observations, there seems to be a decrease in the size of the parathyroid as a result of inanition. When the average diameters and corresponding (final) body-weights are plotted graphically, the parathyroids appear approximately normal. Their decrease during inanition therefore would appear to be nearly proportional to that of the whole body.

In histological structure, the parathyroids in adult rats after acute and chronic inanition show changes essentially like those described for the younger maintenance rats. The changes are in general less marked than in the thyroid, but there is considerable individual variation. Although there is cell-shrinkage in some places, especially where degeneration is marked, in other places there appears no decrease in the average size of the parathyroid cells.

The cytoplasm in adult parathyroids following inanition sometimes appears nearly normal, but usually shows typical vacuolization ('hydropic degeneration'), or in some cases a more deeply-staining homogeneous or coarsely granular structure (especially accompanying markedly pycnotic nuclei).

The nuclei may be nearly normal, but usually show hyperchromatic changes (more rarely hypochromatic), being usually found in the earlier (rarely later) stages of karyopycnosis. These changes occur not merely in restricted areas, or near the free surface (as found in the normal gland) but usually and typically involve large areas and frequently the entire gland. Thus while no new types of cells occur, the atrophic types which occur infrequently and in small amount in the normal gland become very frequent and extensive during inanition. The changes in general appear to be somewhat more marked and extensive during chronic than during acute inanition, although there are individual variations.

The interstitial connective tissue or stroma of the parathyroid appears usually somewhat more distinct, and often relatively increased slightly in amount, during acute and chronic inanition of adults.

d. Discussion and conclusions

Broadly speaking, we may conclude from the foregoing that in general the effects of inanition upon the parathyroid gland are similar to those on the thyroid, but somewhat less marked. The similarities and differences include the following.

The parathyroid gland apparently belongs to that group of organs which in young rats tend to continue growth, even when the body-weight is held constant. This group includes the eyeballs, spinal cord, testes, skeleton, alimentary canal, suprarenal glands and hypophysis. The thyroid, on the contrary, belongs to the group losing weight (Jackson '15 b).

In the parathyroid, many of the individual cells apparently do not decrease in size during inanition; whereas in the thyroid the decrease is usually well marked, especially in the cytoplasm.

In both parathyroid and thyroid glands, the cytoplasm usually shows marked changes during inanition, whether or not it is decreased in amount. Vacuolization ('hydropic degeneration') is most frequent, but deeply-staining, eosinophile condensations of homogeneous (especially in the thyroid) or coarsely granular (especially in the parathyroid) types may occur.

The nucleus in both parathyroid and thyroid glands during inanition tends to become hyperchromatic (more rarely hypochromatic). Thus the nuclei are commonly found in various stages of pycnosis, the earlier stages being typical in the parathyroid, while in the thyroid the later stages (and even karyorrhexis) may frequently occur.

In both parathyroid and thyroid (especially the latter) the normal structure is somewhat variable, and a few cells of atrophic, degenerative character occur. These atrophic types become much more pronounced and numerous during inanition.

The fibrous interstitial tissue (stroma) undergoes little change during inanition, though sometimes it is increased in amount by edemic infiltration (especially in the thyroid).

Very little is found in the literature concerning the effect of inanition upon the parathyroid glands. Erdheim ('03) finds in the human parathyroid small droplets or granules of a fatty nature, which increase in number with age, and are apparently independent of the general nutritive condition of the body.

Pepere ('06) in his extensive work on the parathyroid gland mentions briefly (p. 265) some observations upon the effects of inanition on the parathyroid in dogs starved 9 to 27 days, and in two human cases of death from inanition following oesophageal obstruction. The parathyroid is found relatively less affected than any other viscera. In the dog there is atrophy of the parenchyma, especially of the cytoplasm, which becomes greatly reduced in amount, with vacuolization and loss of the characteristic granulation. The nucleus is hyperchromatic. In the human parathyroid, the effects of inanition are similar, including: "rimpicciolimento dei corpi cellulari, diminuite le granulazioni protoplasmatiche, scarsissimi gli elementi cromofili, estese degenerazioni idropiche e vacuolari delle cellule, assente la colloide." Thus Pepere's observations upon the human and canine parathyroids are in general agreement with my findings in the rat, excepting that he does not mention the occurrence of (advanced) pycnosis or karyolysis.

According to Thompson ('06) the parathyroids in man cannot be said to have a distinct pathology, although various degenerations and other pathologic conditions have been described. He cites the work of Peterson and of Benjamins, in which parenchymatous atrophy, hydropic degeneration, etc., are shown to occur frequently in the human parathyroid.

Following the description by Welsh ('98) there are generally described in the parathyroid of man (and some of the lower animals) two types of cells: (1) 'principal' cells described by Welsh as having in man "a relatively small and clear protoplasmic body, and a relatively large and clear nucleus." These cells constitute the chief part of the gland, and are constantly present. (2) The 'oxyphile' cells usually have relatively more cytoplasm, finely granular and sometimes finely vacuolized. The nucleus is both relatively and absolutely smaller than in the 'principal' cells, its outline more circular and chromatin more dense, "so that no nuclear network can be detected and the entire nuclear mass appears uniformly and deeply stained." These 'oxyphile' cells are found by Welsh in a very large proportion of cases, though not all, and never so abundantly as the 'principal' cells. They never form a large proportion of the gland, and may occur as small masses (often just beneath the capsule) or as scattered cells throughout the gland.

The presence of these 'oxyphile' cells has been confirmed in man and a part (not all) of the lower animals. They are generally considered to represent cells in functional activity. Against this view, however, is the fact that they are quite variable in appearance, and frequently absent altogether (sometimes in man; constantly in some of the lower animals, and especially in the young).

Although I have made no special study of the parathyroid in any other form, the conditions found in the rat lead me to suggest the possibility that these 'oxyphile' cells may represent merely atrophic types, with no functional significance. It will be noted that (although the cytoplasm is relatively abundant) the nucleus of these 'oxyphile' cells as described by Welsh and others corresponds closely with the pycnotic condition characteristic of atrophic, degenerated cells, such as are described above in the normal parathyroid and thyroid glands, and are increased in number during inanition. While the evidence at hand is insufficient for a final conclusion, it seems to me that this interpretation is a possibility which should be kept in mind during further investigation of the matter.

SUMMARY

The more important results of the present investigation may be summarized as follows.

In young rats held at maintenance for several weeks (and hence in a condition of chronic inanition), the histological changes in the thyroid are varied. The follicular epithelium is atrophied, with reduction in height. The nuclei are rarely hypochromatic (various stage of karyolysis), but hyperchromatosis is more typical, the nuclei usually presenting some stage of pycnosis. In the earlier stages the nucleus may be nearly normal in size and structure, excepting a pale, homogeneous coloration of the nuclear background. In more advanced stages, the nucleus diminishes in size, with deepened coloration, forming a dense, deeply-staining, homogeneous mass (typical pycnosis). In extreme cases the nucleus becomes fragmented (karyorrhexis). Neither mitosis nor amitosis is found.

The cytoplasm is usually reduced in amount considerably more than the nucleus. The cytoplasm may show no marked change in structure (simple atrophy), but usually becomes rarefied, with a marked vacuolization ('hydropic degeneration') and loss of the normal granulation. This is especially marked in the few cells where the cytoplasm has lost but little in volume. In some cases the cytoplasm may become homogeneous ('colloid' type) and in advanced stages may disintegrate, forming irregular, deeply-staining (eosinophile) masses of varied appearance.

The intrafollicular colloid may show no abnormal changes. Advanced stages of degeneration in the follicular epithelium, however, are accompanied by dissolution and disintegration of the colloid. The colloid is often replaced by desquamated epithelial cells in various stages of degeneration, and the entire follicle may collapse into an irregular mass.

The interfollicular connective tissue (stroma) usually shows no very marked change in structure, but is often increased in volume by an infiltration of ground substance, giving a somewhat edemic appearance. On this account, the whole thyroid gland may show but little loss in absolute weight, although there has been a marked atrophy of the parenchyma.

In the adult rats subjected to acute and chronic inanition, the changes in the structure of the thyroid gland are likewise varied, but in general similar to those found in the younger rats. The interpretation of the changes in the older rats is more difficult, on account of the frequent occurrence in the normal (control) rats of degenerative changes somewhat similar to those found in advanced stages of inanition.

These changes, involving desquamation and degeneration of the follicular epithelium, have frequently been observed in the thyroids of rats both normal and under various abnormal conditions. They also occur as pathological changes in various other glands. It is suggested that the similarity of these cell-changes may possibly be due to cell-inanition as a common underlying factor.

The parathyroid glands appear to be relatively larger in the female. They apparently belong to that group of organs in which growth persists in young rats, even when held at maintenance (constant body weight) by underfeeding. In adult rats during acute and chronic inanition, the reduction in the size of the parathyroids is nearly proportional to that of the body as a whole.

In histological structure, the parathyroid gland is relatively more resistant than the thyroid to inanition. The changes in the structure of the epithelial cells are somewhat similar to those described for the thyroid, though in general less marked. In many of the cells there is apparently no decrease in the average size, but some (especially those degenerated) show marked shrinkage. The nuclei may remain nearly normal in size and structure, though usually exhibiting various stages of (rarely) karyolysis or (more frequently) karyopycnosis. No cell-division is found. The cytoplasm may be either somewhat reduced in amount, sometimes deeply-staining ('oxyphile'), or may remain nearly normal in volume, with marked vacuolization ('hydropic degeneration'). The stroma may remain normal in amount, but is occasionally increased in volume by infiltration of ground substance.

Variations also occur normally in the structure of the parathyroid (though less marked than in the thyroid), so that here likewise caution must be observed in drawing conclusions as to effects of experiments. The 'oxyphile' type of cell does not appear to be a normal constituent of the parathyroid gland in the rat. However, some of the degenerated cells resemble in many respects the usual description of this 'oxyphile' type, which therefore may possibly represent an atrophic rather than a functional condition.

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EMBRYOLOGY AND MEDICINE

By

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MINNEAPOLIS

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EMBRYOLOGY AND MEDICINE*

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The relation of anatomy to medicine is clearly recognized. A knowledge of the normal human mechanism is an evident necessity, if its abnormal conditions are to be understood and controlled. But the human body is something more than an ordinary machine: it is a machine with a history. To know the machine you must know its history. This history, which includes all of the stages from the formation and union of the germ-cells up to the adult body, is termed *embryology.*

Embryology is therefore of great importance for medicine (1) because it enables us to understand the *normal* adult mechanism; and (2) because it furnishes a rational basis for the prevention or control of *abnormalities*, which may arise at various stages. Let us briefly consider each of these reasons.

The human adult presents a mechanism of almost inconceivable complexity. When we consider it as a cell-colony, however, it appears greatly simplified. And embryology forms a chief support of the cell-theory. No matter how large the number of cells nor how great their modifications in the adult organism, we learn through embryology that in every case they arise ultimately from a single cell, the fertilized ovum, from which by division and differentiation the adult tissues and organs are gradually formed. Thus embryology may be said to present the key to adult structure.

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Embryology, however, furnishes the key, not only to the *normal* structure, but to many *abnormalities* which occur. This phase of embryology is especially important to the physician, whose problem is the prevention and control of vital abnormality, that is, disease. Now, the factors which control the development, normal or abnormal, of the organism may be divided into two fundamental groups: the intrinsic, or hereditary, and the extrinsic, or environmental.

The intrinsic, or hereditary, factors are represented by the organization of the germ-plasm, formed by the union of the paternal spermatozoön with the maternal ovum. The paramount importance of heredity in determining the developmental process is apparent, though clearly recognized only in recent times. The potency of heredity, as compared with environment, in moulding the destiny of living organisms, is now a biological axiom. Consider what determines whether a given ovum will develop into a plant or an animal; an invertebrate or a vertebrate; a fish or a fowl; a dog or a man. In all these cases we must admit that the primary characteristics of the organism are determined by heredity, and cannot be changed by altering the environment. Even individual characteristics, such as sex, stature, pigment of skin, hair and eyes, are also in large measure similarly predetermined.

The supreme influence of heredity is evident also in the mental makeup of every individual.

We know that, in a general way, mental ability and temperament are inherited. You are all doubtless familiar with the ancient doctrine of the temperaments, associated with the "humoral pathology," which dates back to the time of Galen, or earlier. Though largely discredited, this doctrine contains an element of truth, as would appear from the recent investigations of Davenport. He suggests that there are in the germ-plasm factors representing various grades of hyperkinesis (calm, nervous, choleric) and of hypokinesis (cheerful, phlegmatic, melancholic). These factors behave as though in different chromosomes, and appear to be inherited independently, so they may occur in any combination. Thus mental characteristics, as well as physical constitution, are largely determined by heredity.

Heredity also plays an important part in the occurrence of disease. In some cases, defects, such as color-blindness and congenital hemophilia, are undoubtedly hereditary in character. This applies also to a large percentage of cases of insanity and to many other disorders of the nervous system and sense-organs. In the light of our present knowledge, however, it appears that what is transmitted by heredity is usually not the disease as such, but *constitutional predisposition* to disease,—for example, cancer, tuberculosis. Evidence is accumulating to show that the hereditary transmission of such predisposition (diathesis), even in cases where the immediate cause is an environmental factor, is more important than has hitherto been generally realized.

In many cases, doubtless, both heredity and environment thus share as factors in the production of disease. In the past, however, attention has apparently been focussed too exclusively upon the environment as the cause of disease. Although this has resulted in great progress in the field of the infectious diseases, other disorders in which the hereditary factors are more prominent have been relatively neglected. Thus the practical importance of a knowledge of heredity is becoming more clearly recognized as essential to the physician; indeed, eugenics will probably play a large part in the preventive medicine of the future. The rapid advances in the science of heredity are admirably presented in the recent book by Conklin on "Heredity and Environment" (Princeton University Press, 1915).

While from the biological point of view we must admit the predominance of the intrinsic or hereditary factors, still from the medical point of view we must also recognize the great importance of environment, especially in the causation of disease. While it is true that the bounds of possibilities in development are rigidly set by heredity, yet it is equally true that within these limits the process is controlled by environment. Since an unfavorable environment may, in countless ways and in various degrees, modify, limit, retard, or altogether prevent the process of development, it might well be argued that, from the point of view of pathology, environment is even more important than heredity.

In this connection we must bear in mind that environment is not limited to postnatal life. It is a fact not sufficiently recognized that prenatal environment is of even greater importance. We sometimes forget that the individual begins its existence not at birth but as the fertilized ovum. And it is pre-eminently during the earlier stages of development that changes in the environment are especially effective in producing disturbances in the delicately balanced organism. The available evidence indicates that practically all the monstrosities are produced at this time by changes in the chemical or, more rarely, physical environment. These changes may be due to faulty implantation of the ovum in an unhealthy uterine mucosa, or they may be due to various organic or inorganic toxins from the maternal circulation. Imperfect respiration and nutrition, on account of placental defects, may also play an important part in disturbances of the developmental process. While, broadly speaking, the organism apparently becomes more resistant as development proceeds, yet each stage appears to have its special points of weakness and liability to disorder. This is well known for the various postnatal stages of infancy, childhood, and puberty; and it is probably equally true for prenatal stages. A better knowledge of the developmental process thus becomes a necessity for advancement in prenatal pathology and hygiene.

In considering the process of development with the various associated abnormalities, it is important to remember that the essential features are due to changes in the component tissues and cells. The development of the organism with its complicated parts is merely the visible resultant of the growth and differentiation going on in its myriads of constituent cells.

These cell-changes have been summarized in Minot's doctrine of "cytomorphosis," according to which every cell during its life-history goes successively through stages of growth and differentiation, followed sooner or later by degeneration with cell-death and removal. The "how" and "why" of this process of cell-differentiation is the fundamental problem of embryology. What causes one embryonic cell to become a gland-cell, while another becomes a muscle-cell or a bone-cell? What makes cells divide? What are the cell-changes underlying the progressive developmental stages of the embryo, fetus, new-born, infant, child, adolescent, and adult? The solution of these problems will give us a deeper insight into not only the normal cell-structure and activity but also the causes of the various types of departure from the normal. We may then be able to understand the arrest or distortion of the developmental cell-processes, producing the various organic defects and associated functional derangements. In short, these fundamental biological and medical problems are ultimately and essentially cell-problems.

Associated with, and yet distinct from, the process of embryonic differentiation is the process of growth, a subject in which I have been especially interested. More closely associated with growth is the process of cell-division. Both cell-growth and cell-division are most rapid and vigorous in the early embryonic stages, when cell-differentiation has scarcely begun; while later, when cell-differentiation becomes prominent, the processes of cell-division and cell-growth become progressively much slower. Thus, while the early embryo may double its mass in a few hours, the growth-rate decreases rapidly, so that at birth it requires about five months for the infant to double its weight. Thereafter the growth-rate continues to decrease, with an exceptional acceleration at puberty, to zero in the adult.

The growth-rate, however, is not uniform throughout the whole body. In accordance with what I have termed the "law of developmental direction," the region of greatest intensity of growth shifts in a general cephalocaudal direction. As a result, the head region is relatively largest in the early embryo, while the pelvis and lower extremities reach their greatest relative size in the adult. In any given region of the body there is, furthermore, a dorsoventral path of growth, the dorsal structures in general preceding the ventral in development.

A further analysis reveals the fact that, even in any given region, there is considerable diversity of growth-rate in the various organs. Each has its own characteristic curve of growth. Some organs (brain, liver, suprarenals) reach their maximum relative size in the embryo; other (kidney, spleen) later in the fetus; while still others (musculature) may not reach their relative maximum until in the adult stage. Similarly, within each organ we find varying growth-rates in the component parts, tissues, and cells at difrent stages.

Moreover, the normal growth-rates of the various tissues and organs are changed in characteristic ways under abnormal conditions. I have recently studied the effects of inanition upon the growth of the body and of the various organs of the white rat. When weaned, at the age of three weeks, the rats were put on a restricted diet, and held at nearly constant bodyweight for several weeks or months, until the control rats on full diet were about half-grown. In the retarded rats, the growth-power is not lost, but merely suspended; for on re-feeding they grow rapidly, at a rate even above the normal, so they soon overtake the controls. Whether it is possible to carry the process of starvation to a point where no growth results on re-feeding

TABLE OF SECTIONED HUMAN EMBRYOS.

No. and sex	Crown-rump length mm.	Fixation	Sections micra	Donor	Remarks
H 1	15	?	t 12		Good.
H 2	20	Formalin	t 15	Dr. Dart	Good.
H 3	22	Zenker	t 12		Good.
H 4	8	?	?		Abnormal.
H 5 m	25	Zenker	t 15		Fair.
H 6	6	Zenker	t 10	Dr. Schludt	Fair, Incomplete.
H 7 m	20	?	t 10		Fair.
H 8 m	41	Formalin	t 12	Dr. Cheney	Excellent.
H 10 m	29	Alcohol	t 15		Fair.
H 11 m	60	Formalin	t 33	Dr. Tebbits	Poor.
H 12 f	41	Zenker	t 15		Excellent, Damaged.
H 13	7.5	Alcohol	t 15		Good.
H 14	12	?	t 10		Fair.
H 15	22	Alcohol	t 12		Good.
H 16 f	33	Zenker	t 15		Excellent.
H 17	7	Alcohol	t 10		Fair.
H 18	15.5	Formalin	t 10		Excellent.
H 19	22	Formalin	t 12		Ectopic, Poor.
H 20	6	Picro-sulph.	t 8	Dr. Cates	Poor.
H 21 f	26	Alcohol	t 15	Dr. Rose	Excellent.
H 22	19	Picro-sulph.	t 12		Good.
H 23	15	Alcohol	t 10		Fair.
H 24	19	Zenker	t 12		Fair.
H 25	2.5	Alcohol	t 8	Dr. Coleman	Poor.
H 26 m	65	Formalin	t 30-50		Fair.
H 28	16	Alcohol	t 10	Dr. Abbott	Ectopic, Normal.

H 51 m	35	Formalin	s 50-200	Drs. Milnes and Jackson	Irregular.
H 54 f	150	Formalin	c 50-t 100	Dr. Jackson	Head (c); trunk (t) (inc.).
H 55 m	65	Formalin ?	t 45-50	Drs. Hume and Jackson	Fair.
H 56 f	24	Alcohol	t 20	Drs. Scholz and Jackson	Good.
H 57 f	31	Formalin	t 20	Drs. Graham and Jackson	Good.
H 58 f?	17	Formalin	t 20	Drs. Douglas and Jackson	Excellent.
H 60	11	Alcohol	t 20	Drs. Lewis and Jackson	Excellent.
H 61	6	Formalin	t 10	Drs. Kuhn and Jackson	Abnormal.
H 62	16	Formalin	s 20 ?	Drs. Pond and Jackson	Fair.
H 63	18	Alc.-form.	t 20	Drs. Douglas and Jackson	Poor.
H 64 m	23	Alcohol	s 20 ?	Drs. Ryland and Jackson	Fair.
H 65	7.3	Alcohol	t 10	Drs. Vandeventer and Jackson	Poor.
H 67	5	?	s 10		Poor.
H 68	11	Bouin	t 15	Dr. Johnson	Excellent.
H 79	1-2 ?	Alcohol	8	Dr. Conley	Ovum, Abnormal.
H 87 f	65	Formalin	s 50	Dr. Bray	Unfinished.
H 97	14	Alc.-form.	t 20	Drs. Lewis and Jackson	Abnormal.
H 98 m	30	Bouin	s 15	Dr. Adair	Good.
H 99 f	26	Alc.-form.	t 20 ?	Drs. Graham and Jackson	Fair.
H 100 f	43	Formalin	s 20	Dr. Thompson	Good.
H 102 f	95	Formalin	s 50	Dr. Potter	Unfinished.
H 108 f	30	Zenker	t 10	Drs. Norris and Jackson	Head damaged.
H 115 m	50	?	s 40	Drs. Anler and Jackson	Fair.
H 121 m	46	Formalin	t 60	Drs. Herndon and Jackson	Fair.
H 122 m	39	Formalin	t 20 ?	Drs. Herndon and Jackson	Fair.
H 134	12	Formalin	t 20	Dr. Clark	Fair.
H 203 m	177	Formalin	s 100	Dr. Potter	Unfinished.
H 259 f	30	Formalin	t 20	Dr. Adair	Fair.
H 260	18	Formalin	t 15	Dr. Phelps	Good.
H 266 m	168	Formalin	s 100	Dr. Potter	Unfinished.
H 275	183	Zenker	s 100	Dr. Johnson	Unfinished.
H 277	2 ?	Alc.-form.	t 8	Dr. Adair's collection	Pathological.
H 278	2 ?	Formalin	t 8	Dr. Adair's collection	Pathological.
H 279	4 ?	Formalin	t 8	Dr. Adair's collection	Pathological.
H 280	13 ?	Bouin	Unfinished	Dr. Adair's collection	Pathological.
H 281	10 ?	Formalin	Unfinished	Dr. Adair's collection	Pathological.

is uncertain, although Aron has apparently obtained permanent stunting in this way.

In young rats thus held at constant body-weight, the effects differ greatly upon the different organs and systems. Some organs (skin, spleen, thymus) lose markedly in weight; others (musculature and most of the viscera) remain nearly stationary; while in a third group (including skeleton, spinal cord, and eyeballs) the growth-impulse is so strong that they increase in weight at the expense of other organs, even when the body-weight is held constant. The practical bearings of these results are obvious, since similar relations probably obtain in the human species. While much of importance is known, far more remains to be discovered concerning the whole process of development, from ovum to adult, before we can be fully prepared to detect and control abnormal conditions.

Admitting the necessity for further research in the field of embryology, the question arises as to the best method by which this may be accomplished. Here, as in science generally, we may follow either of two methods: the *descriptive* method, by which we observe the phenomena as they occur in nature; or the *experimental* method, by which we attempt to get a deeper insight into the phenomena by varying the conditions and observing the effect. Each method has its advantages and its limitations. Generally speaking, science progresses most rapidly by utilizing a combination or alternation of the two. The descriptive method reveals the phenomena; the experimental method gives insight into their nature and cause. Thus in descriptive embryology we learn the morphology of the organism and its constituent parts at various stages of development. With this knowledge as a basis, by experimental embryology we seek to learn more of the significance of these phenomena, their cause and methods of control. In experimental embryology, the results are often more brilliant, and the future possibilities are very promising; but very much yet remains to be done in the field of purely descriptive embryology. Even among the lower forms, where abundant material is easily obtained, many important stages are yet undescribed.

In human embryology, the gaps in our knowledge are even wider and more numerous, as one may realize in looking through the excellent recent manual by Keibel and Mall. In many cases, the earlier stages of organogenesis and histogenesis have been fairly carefully worked out, and the adult structure is well known, while the intermediate changes in the fetus and child remain undiscovered. My colleague, Professor Scammon, is especially interested in this intermediate field, and has organized a much-needed course in "Fetal and Newborn Anatomy" for advanced and graduate students. In general, it may be said without exaggeration that with adequate material almost every phase of human embryology could be studied or restudied with great profit to biology in general and to medicine in particular.

Lack of adequate material is perhaps the greatest drawback to progress in embryology at present. There ought to be a great collection of embryos available for study at every university. There are, however, at present but three or four noteworthy collections in this country. The collection of human embryos by Professor Mall at Johns Hopkins and that of vertebrate embryos by the late Professor Minot at Harvard are the largest and best of their kind in the world, and have been of great service. At the University of Minnesota we have made (largely through the efforts of my colleague, Professor Lee) a fairly good beginning, with a collection in both human and comparative embryology.

In the comparative collection, we have now 579 sectioned embryos of various species in different stages. They are all cut in serial sections, stained, and mounted ready for study. The following list indicates the forms, with the number of series available at present in each:

Fishes:	
Petromyzon	33
Squalus	27
Amia	51
Ameiurus	10
Salmo	3
Micropterus	18
Amphibians:	
Amblystoma	80
Necturus	11
Rana	40
Bufo	19
Hyla	17
Reptiles:	
Tropidonotus	5
Vipera	4
Anguis	3
Lacerta	14
Chelydra	18
Chrysemys	1
Birds:	
Gallus	76

Mammals:

Mus (norv.)	3
Sus	120
Ovis	4
Lepus	2

In the department collection of human embryos, we have at present about 300 specimens. including various stages from the earliest up to full term. Of these, 75 have been selected and cut into serial microscopic sections, as indicated in the following table. Following the number, m= male; f= female. The length is the crown-rump length in millimeters. The thickness of the sections is given in micra. The letter t indicates that the sections are cut in the transverse plane; s, sagittal; and c, coronal.

In addition to the general department collection, I may mention also certain special collections made by members of the staff and equally available to others for purposes of research. Professor Scammon has a series of about 60 Elasmobranch embryos, representing 9 species, of various stages in serial sections. Professor Lee has collected a series of about 400 mammalian embryos, chiefly rodents, representing 15 genera, mostly early stages cut *in situ* with uterus, and especially valuable for investigation of the early embryo, implantation, membranes, etc. Professor Johnston has an extensive neurological collection, including about 200 sectioned embryos of fishes and amphibians (Amia, Ameiurus, Lepidosteus, and Amblystoma), also a series including 15 stages in the development of the human brain (Flechsig's method) up to the new-born.

I take this occasion to thank most heartily the numerous physicians who have kindly contributed the specimens in this collection. Permit me to point out to you that here is an excellent opportunity for the physician to render a valuable aid by preserving and forwarding to the laboratory any specimens which he may obtain. The laboratory man is not in medical practice, so the progress of our knowledge of human embryology depends largely upon the interest and co-operation of the practitioner. All specimens, normal or abnormal, will be gratefully received, and placed in the collection. Containers with preserving fluid (10 per cent formalin is best for general purposes) and return postage will be sent upon request. A brief clinical history, including the last menstrual date, is urgently requested, and will add greatly to the value of the specimen. We will gladly make report if desired as to the probable age of the embryo, pathological condition, etc.

The collection is rapidly growing, and with your co-operation we hope to make it equal to the best. Our laboratory is always open to any of you who may desire to use its collections and other facilities for research, and we will also upon request loan the specimens to any qualified investigator for study elsewhere. It is our hope that in this way the collection may serve a valuable purpose in promoting research work in embryology, and thereby contribute to the advancement of medicine.

DISCUSSION

Dr. T. G. Lee (Minneapolis): Dr. Jackson has covered the general scope of the topic under discussion so thoroughly and so well that little remains to be added.

In the same way that the older ideas regarding the structure of the body, dating back to the time of Galen.

were revolutionized and purely theoretical considerations replaced by *fact*, due to the work of Vesalius and his successors, so in more recent times it is through the youngest daughter of Anatomy, namely, Embryology, that we are at last coming to a proper understanding and interpretation of the mass of data which has accumulated relating to the structure of the human body,—man's place in nature, his relationship to other forms of life, and many of the otherwise obscure problems of physiology and pathology.

Embryology is a recent science. It is less than one hundred years (1827) since Karl von Baer laid the foundation of modern embryology in his classical work in which he said that "Embryology is the torch bearer in the study of all organic forms." The truth of that statement has become year by year more certain. For years embryology did not form a definite part of the medical-school curriculum; and, when it was studied, the chick was the form commonly used. This was natural. Collections of mammalian embryos were to be found in but few laboratories, and human embryonic series were still more rare. Two methods of study have been employed: one, that of tracing out step by step the formation and growth of organs and body of a selected type as chick, frog, etc.; the other method, that of tracing the development of a given organ or part in as many different forms as possible all through the vertebrate scale. Both methods have yielded splendid contributions to our knowledge of the subject, and shed much light on human development. We must not forget that there still remain very important gaps in our observational knowledge of human growth: no one has yet seen segmenting human ova, the details of the passage into the uterine cavity and implanation of ovum, the differentiation of the germ layers, the formation of the fetal envelopes, and other important facts are still unrecorded for the human subject. Thus we are still dependent on comparative material. Until some fifteen years ago it was supposed that the human ovum attached itself to the surface of the uterine mucous membrane, and then placentation was originated; but by the rare good fortune of being able to make an immediate autopsy on a girl who suicided in a Viennese Hospital, Dr. Peters was able to secure what was at that time the youngest known human embryo. This specimen demonstrated that the previously held view of the formation of the placenta was erroneous, and, instead, that the ovum perforated the epithelial layer and formed a new decidual cavity in the uterine connective tissue. Embryologists at once saw that here was something similar to the previously described method of implantation of the ovum in the guinea-pig, which was unique for mammals as then known, and that in all probability the guinea-pig would throw light on the unknown steps in man. This illustrates the importance of the study of other forms as applied to human development. Since then it has been our good fortune to find two other mammals with this same type of implantation

If we are ever to be free from our dependence on the study of other animal forms in teaching human embryology, it will be when the profession realize that the laboratory men are entirely dependent on them for the needed material. Thousands of embryos are lost every year, which, if saved, would aid in solving many of the still unknown or unsettled problems of human development.

Dr. Jackson has pointed out many of the important applications of embryology to medicine, and Dr. Wilson, who follows me, will be able to give numerous examples of the importance of a knowledge of embryology in surgery and pathology.

The question that has been repeatedly put to each of you here by some anxious parent has been, "Is it a boy or a girl?" Many of you have had put to you that other question by an anxious patient, "Will it be a boy or a girl?" The laboratory man is constantly asking himself, "Why and how does it become a boy or a girl?"

We all grow from a single microscopic cell. We all change from a common indifferent stage to one or the other sex for reasons still unknown. These and many other important problems in medicine can be at last solved by the laboratory worker, if you will do your duty, and furnish him with the needed material.

Dr. L. B. Wilson (Rochester): We are all inclined to think of the embryologist as a man surrounded with subject matter that is very dead. But, if we could attend the annual meeting of the Association of American Anatomists,—for there is reported the work of the embryologists,—we would find their studies very much alive. The reason for this lies largely in the fact that the embryologists are going back to the beginning of things.

A great many theories in medicine are based upon supposed facts that are clearly "hot air." As a profession we are still taking our hats off to many that have absolutely no basis in fact. And many of us are incapable of determining whether the support of the theories we approve of are facts or not. So far as morphology is concerned the anatomist and embryologist is capable of making such a determination, and he is doing so with a high degree of accuracy.

What are we, as a profession, doing to aid in his work? The truth, which Dr. Lee tells us, is most regrettable, that never yet has there been placed in a scientific collection a human embryo in the earliest stages of development. Gentlemen, we are like South African savages feeding diamonds to their pet ostriches. We have thrown away thousands of the finest embryologic specimens. We should at least sufficiently feel our responsibilities to the science of medicine to save the embryologic material that we are now destroying. That is the plea Dr. Jackson is making to the medical profession in Minnesota, and I wish to second that plea in the most emphatic manner.

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EVIDENCE OF A MOTOR PALLIUM IN THE FORE-BRAIN OF REPTILES[1]

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ONE FIGURE

At the title indicates, the purpose of this note is not to describe the structure of the reptilian motor cortex nor to discuss the localization of function within this area. The purpose is only to give such evidence as is now at hand that a specialized area comparable to the mammalian motor cortex probably exists in the reptilian brain and to indicate the general position and extent of this area.

The writer has indicated in a previous paper ('15 b) that the rostral portion of the dorsal pallium in the turtle differs structurally from the rest and has suggested the possibility that this area may correspond to the motor cortical area in the mammalian brain.

This hypothesis is being tested by degeneration methods and by cortical stimulation. Sufficiently definite results have been obtained by the latter method to indicate that motor and sensory fields can be distinguished in the reptilian pallium.

Three species of turtle (Chelydra serpentina, Cistudo carolina, and Chrysemys marginata) and one lizard (Gerrhonotus) have been studied. Two methods of stimulation were used: induction shocks with two-point electrodes, the two points closely approximated; induction shocks with one point electrode, the second electrode being formed by a copper plate covered with moist cloth on which the body of the animal rested.

An important factor in the experiments is the degree of anaesthesia employed. The clearest results have been obtained with very deep anaesthesia. Some of the turtles were given a dose of morphine by hypodermic injection, and all were anaesthetized by means of chloroform placed on cotton in the mouth or injected directly into the trachea or both. Although the resistance of these animals was well known, in several cases the anaesthesia was not carried far enough and the reactions to cortical stimulation were prolonged contractions of neck muscles or struggling movements of the limbs indicative of pain. In these cases occasional responses, as of the eye muscles or temporal muscle, appeared to be due to local excitation of the cortex but the more extensive and prolonged movements were elicited from any part of the cortex and were not distinguishable from the responses to stimulation of the dura mater, the lower brain regions or even the tissues exposed in the head. Under conditions of deep anaesthesia the responses consisted of contraction of a small set of muscles, and of short duration, and these were obtained from a certain region of the pallium only. The lizards are not so difficult to anaesthetize, but long continued application of chloroform is necessary (one and a half to two hours in a closed dish containing a wad of cotton wet in chloroform).

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[1] Neurological Studies, University of Minnesota, no. 22, June 29, 1916.

Another factor presenting difficulty is the small size of the brain in these animals. This makes it necessary to use a single point electrode or to have the two points very close together. It is of the greatest importance that the meninges be carefully removed from the whole hemisphere and that the brain and surrounding tissues be kept as dry as possible without injury to the brain tissue. If the electrode comes too close to the dura mater or if fluid facilitates the spread of the current to the dura, the muscles, thalamus or midbrain, the results are vitiated because of responses coming from two or more sources of stimulation. The responses which follow stimulation of the dura or brain stem are very different from those produced by cortical excitation, being usually more vigorous and prolonged as well as more extensive. In the lizards and in several turtles, after the dura was laid back and the olfactory and optic connections were cut, the entire forebrain was raised out of the skull so as to be free from contact with other tissues. Under these conditions the entire surface of the hemisphere was explored with the electrodes.

With the induced current minimal stimuli were applied at first and increased later in the experiment when it became necessary in order to obtain responses. In many cases the brain fatigued quickly and in some it recovered with equal promptness. Individuals differed greatly as to the recovery. Often when the strength of stimulus was increased struggling movements resulted and the experiment had to be discontinued. In some cases also, the animals gave definitely localized responses to the first stimulation and later, probably because of recovery from the anaesthesia, gave irregular and prolonged reactions.

CORTICAL REACTIONS IN TURTLES

If we take into account only the short contraction of restricted groups of muscles, the following parts of the hemisphere have been found to give muscular responses: dorsal surface of the olfactory bulb, retraction of the neck, extension of the legs, movements of eyeball and eyelid; dorsal surface of pallium near olfactory peduncle and lateral border of pallium in the anterior one-half or two-thirds of hemisphere, movements of eyes, jaw, neck, legs and tail; striatal area, movements of all parts. No responses were obtained from any other part of the dorsal surface or the medial wall or from the tuberculum olfactorium, the amygdaloid eminence or elsewhere, except that contractions typical of thalamic stimulation were sometimes obtained when the caudal pole or amygdaloid eminence was being explored. These were probably due to spread of current to the thalamus.

The responses from the striatal area were presumably due to the direct stimulation of descending fibers in the crus. The responses from the olfactory bulb may be due to the close proximity of the 'motor area' which is indeed overlapped by the caudal border of the olfactory formation. Thus it appears that a somewhat comma-shaped area involving the rostral and lateral borders of the general pallium (fig. 1) may be regarded as a motor area in the turtle's pallium. This area corresponds roughly to the 'pallial thickening' described in a previous paper ('15 b).

Not enough experiments have been conducted under uniform conditions to furnish a basis for the discussion of localization within this area. As the whole area is only a few millimeters in extent, it is obvious that great care must be exercised in accepting as conclusive any apparent indications of special function of particular parts of this area. For this reason I mention only tentatively and with reserve that leg movements have been obtained most often from the anterior part of this area and eye, jaw and neck movements from the lateral portion. Further experiments will be made to determine whether a localization within the motor area clearly exists. In certain experiments the eye and jaw movements were homolateral only or more frequently, while the neck and leg movements were mostly heterolateral.

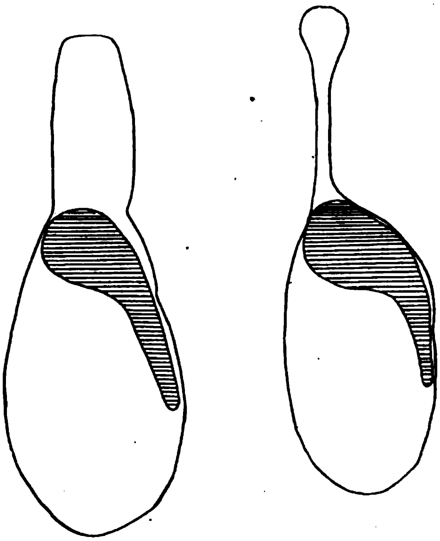

Fig. 1 Sketches of the dorsal surface of the right hemisphere of the turtle (left) and lizard (right) on which the motor area is shaded.

Excitation of the dorsal ventricular ridge in the turtle was carried out by cutting away the whole dorsal pallium and applying the electrodes to the ventricular surface of the ridge. Muscular responses were often obtained, such as retraction of the eyeball, contraction of temporal muscle and twitching of fore leg, but there is the double danger of spread of stimulus to the closely adjacent pallial thickening and of stimulation of the underlying crus.

MOTOR RESPONSES IN THE LIZARD

Only four animals have been examined, two of which gave much clearer and more definite results than those obtained from most of the turtles. From the area shown in the accompanying sketch one animal gave movements of the fore leg, jaw and eye muscles, neck and throat muscles, and anterior body musculature. Stimulation of the olfactory tubercle also caused contraction of small muscles in the throat, which was felt as the head was held in the fingers. Stimulation of the crus in the striatal area produced movements of legs and body muscles. Movements of the fore leg were regularly heterolateral, although homolateral movements occurred occasionally. Just the opposite was true of the eye and jaw muscles. The other animal responded to stimulation of the anterior part of this area by strong contraction of pelvic muscles and movements of the hind legs and by weak movements of the fore legs. Stimulation of the lateral border of the right pallium in this animal produced a definite torsion of the fore part of the body.

Gerrhonotus is a rather large lizard and its brain is fairly satisfactory to work with. The results in these two cases were so definite that I regard them as strong evidence that a motor area exists in the lizard pallium essentially similar in position and extent to that in the turtle.

Early in my studies I examined the brain of a single specimen of Alligator about a foot and a half in length, and obtained motor responses from the anterior part of the dorsal pallium, but I have no written record of this experiment.

From these experiments, together with the study of the structure of the hemisphere already reported, I am strongly inclined to believe not only that reptiles possess a general or somatic pallium which has been in dispute until recently, but also that in that pallium are to be distinguished definite sensory and motor areas in the sense in which those terms are commonly used of the mammalian pallium. Localization within these areas and the significance of these areas in the evolution of the mammalian pallium are subjects for further study.

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THE DEVELOPMENT OF THE DORSAL VENTRICULAR RIDGE IN TURTLES[1]

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TWENTY-SEVEN FIGURES

In the adult turtle (Johnston, '15 b) the large ridge projecting into the ventricle from the lateral wall of the hemisphere has strong fiber connections with the cerebral peduncle and has obvious close relations with the cortical layers of the pallium in the caudal pole. Indeed, in its caudal part this ridge appears to be a fold of the whole thickness of the brain wall and is marked externally by the 'amygdaloid fissure.' Here the cell layers of the general pallium turn in to become continuous with the cell masses of the ridge. It is evident from these facts that the relations of the dorsal ventricular ridge are chiefly with the general pallium. Its position lateral and dorsal to both the caudate and lentiform nuclei shows that it does not belong to the corpus striatum. Its independence from other cell masses in its rostral part gives one the impression (from the study of adult material alone), that the ridge has been formed by infolding from the caudal region ('15 b, p. 417).

The origin of this ridge in the embryo should throw light not only on its relations in the turtle but also on its significance in the evolution of the mammalian brain. I am able to give the main outlines of the development on the basis of a series of early embryos of Chelydra serpentina, for which I am indebted to Dr. C. E. Johnson of this University; and of sections of 17, 20, and 28 mm. embryos of Chrysemys, belonging to the Anatomical Laboratory of Washington University for the loan of which I am indebted to Dr. Edwin A. Baumgartner.

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[1] Neurological Studies, University of Minnesota. No. 23, July 1, 1916.

The material in both cases was beautifully sectioned and stained. The brains of freshwater turtles are so similar that the younger stages of Chelydra can be readily compared with the later stages of Chrysemys for the rather general purposes of this study.

AREAS OF PROLIFERATION IN THE EARLY EMBRYO IN RELATION TO CELL MASSES OF THE ADULT BRAIN

Broadly speaking, three periods are to be recognized in the development of cell masses in the telencephalon: a period of evagination of the hemispheres and formation of indifferent cells by mitosis of the germinal cells; a period of form changes and further proliferation of cells in which the successive formation of functionally ind pendent cell masses is noticeable; and a period during which the growth of fiber bundles and other factors bring about the definitive form of the adult cell masses.

In the first period the wall of the telencephalon consists of a deep layer of spongioblasts and dividing germinal cells, a thick layer of indifferent cells and a superficial clear layer or marginal veil of very varying thickness. The thickest part of the marginal veil forms the floor of the groove along the lower border of the hemisphere, at its junction with the brain stem. This is the course of the future lateral forebrain bundle or crus cerebri. The indifferent cells form a continuous layer with no indication of individual masses.

The second period begins with the appearance of independent cell masses separated by cell-free zones which show in sections as clear lines or areas. Such cell-free zones in adult brains have been commonly regarded as boundary lines between individual cell masses which are presumed to have different functions; i.e., centers or nuclei. Similar cell-free zones in the brains of lower vertebrates have been used in recent years as landmarks between important morphological regions, as the pallial and basal areas. The writer has pointed out the need of caution in interpreting these zones in this connection ('13 b, p. 382). It is now to be noted that the formation of individual masses of cells is dependent upon the proliferation of cells from different regions of the germinal cell layer and upon the proliferation of cells at successive periods of development. Thus the proliferation of cells in the dorsal wall gives rise to pallium, that in the basal wall gives rise to various olfactory centers, corpus striatum, etc. Also, certain centers owe their origin to the proliferation of cells in an early stage, other centers to proliferation in later stages. The former come to lie superficial to the latter and the two are separated by cell-free zones. A further factor is the shifting or spreading of cell masses, whether due to cell-migration or to mechanical forces. These several factors must be taken into account in any attempt to use the embryological method in the study of the significance of cell-grouping in the brain.

CHELYDRA SERPENTINA

In the oldest Chelydra embryo studied, it is possible to recognize several of the important cell masses and fiber bundles of the telencephalon and in part the boundary lines between pallial and basal areas. Examination of the model (figs. 1, 2) and of sections (figs. 6 to 14) shows that the telencephalon and diencephalon are narrow from side to side and high dorso-ventrally. The hemisphere is a simple sac which projects much farther rostrad than caudad. The interventricular foramen is still very large. Behind it, the thin wall which represents the choroid plexus extends nearly half way to the caudal pole. This stage illustrates very clearly, both in its general form and its internal structure, how much the rostral pole of the hemisphere precedes the caudal pole in development. The outer surface of the brain shows few of the landmarks which are seen in later embryos and adults. The stem-hemisphere sulcus is of course the most prominent. The olfactory peduncle is not yet formed, but the broad depression on the lateral surface may represent the beginning of constriction. The fissura prima and diagonal band are recognizable at the medio-basal angle in front of the preoptic recess. There is a shallow furrow over the rostral part of the foramen which may be the beginning of the sulcus fimbrio-dentatus.

The ventricle shows a very sharp ventral groove leading forward from the foramen and much deepened in the region of the future tuberculum olfactorium. The middle ventricular groove is clearly marked in the region rostral to the foramen, while the dorsal groove has not yet appeared.

In spite of the simple form of this brain, the internal structure shows considerable advance in differentiation.

The sections illustrated in figures 6 to 14 can be understood best by beginning with that which passes through the rostral border of the foramen interventriculare (fig. 9). In the medial wall above the foramen are recognized the primordium hippocampi and the fimbria, one of the early fiber tracts to be formed in the embryo. The dorsal wall is occupied by pallium, the medial portion by hippocampal, the lateral portion by general pallium. In the lateral wall the pallium seems to be limited by a ventricular groove and by a cell-free space. This boundary line strongly reminds one of the lateral zona limitans of the selachian and frog brains (Johnston, '11 a). The later embryos show this groove to be the middle ventricular groove of the adult brain and the clear area becomes the cell-free zone separating the dorsal ventricular ridge and the nucleus lentiformis in the adult. In the lower half of the lateral wall the following structures are seen in the figure: an active proliferation which gives rise to the nucleus lentiformis, the nucleus caudatus, the lateral and medial forebrain bundles, the anterior commissure bundle, the fiber layer of the diagonal band, and the lateral olfactory area. Although I have not sufficient stages to enable me to follow the history of these structures, there is evidence that the superficial cell layer which represents the lateral olfactory nucleus has been derived from the layers of indifferent cells in the early embryo and that the mass of the caudate nucleus represents a later proliferation. The embryo shows clearly that the lentiform nucleus is beginning to form when the caudate is already completely formed or nearly so. As the other sections are studied it will become evident that the lentiform nucleus is formed by a proliferation distinctly dorsal to the caudate and that the changes in later development bring it into a position lateral to the caudate. There is some indication of the superficial layer of the olfactory area spreading upward beyond the middle ventricular groove (at the point marked *l.pyr*).

Passing caudad from this level, in the next section drawn (fig. 8) the same structures are to be seen in the lateral wall. The middle ventricular groove has almost disappeared, however, and the proliferating area for the lentiform nucleus is much smaller. The next section drawn (fig. 7) is the last one in which the lentiform proliferation can be recognized and with it the locus of the pallial border. In the lower part of the hemisphere the lateral olfactory area is represented by a large collection of cells closely related to the caudate nucleus and the diagonal band. This is the nucleus of the lateral olfactory tract and the next section shows the close relation of both this and the diagonal band to the stria medullaris. Caudal to this the nucleus of the lateral olfactory tract soon disappears and the hemisphere wall presents the appearance of an undifferentiated pallium, except for the fimbria and the thin choroidal area in the medial wall.

The next section rostrad from the one first described passes through the anterior commissure (fig. 10). The lentiform proliferation appears larger, the diagonal band fibers approach those of the olfacto-hypothalamic system. In figures 9, 10, and those of sections farther rostrad, one of the most noteworthy features is the mass of cells proliferating from the deep layers of the pallium. The medial border of the pallium does not show active proliferation but in the lateral three-fourths many new cells are forming and these appear to be streaming laterad into the thick wall just dorsal to the middle ventricular groove. Indeed the proliferation and streaming of these cells is the active cause for the thickening of this part of the wall and the formation of the middle ventricular groove. Further thickening of this part of the wall deepens this groove and produces the dorsal ventricular groove at the point indicated in figure 9.

In figures 9 to 12 appear important relations of the dorsal border of the olfactory area to the pallium. Here it is quite clear that the superficial layer of cells of the olfactory area extends up on the outer surface of the pallium and that it is accompanied by a special bundle of fibers. The crowding of cells in this stage is such that it is impossible to determine whether the pyriform lobe cells have spread or migrated up from the lateral surface in the basal region or have been formed here by the proliferation which at this stage is giving rise to the pallium. Farther caudad and in later stages the relations are such as to make the former appear more probable. The cells of the pyriform lobe probably all come from the layer of indifferent cells seen in earlier stages and formed by proliferation in the lateral and basal region before the pallial proliferation seen in this stage began. Pallium and pyriform lobe are still visible in a section (fig. 14) through the peduncle in which the olfactory formation appears in the dorsal wall. The olfactory formation extends far back on the dorsal surface, as is already well known.

[CHRYSEMYS

Models were made of the 17 mm. and 28 mm. stages. Most of the chief landmarks of the adult brain are already visible in the 17 mm. embryo (figs. 3, 4, 15, 16, 17). The caudal pole still lags behind the rostral portion in development. The caudal pole projects much farther back than in the oldest Chelydra embryo and in the 28 mm. stage the olfactory area (lobus pyriformis) extends relatively farther back than in the 17 mm. stage. The tuberculum olfactorium is well developed and the ventral groove of the ventricle dips deep into it. The middle ventricular groove (fig. 4 and sections) is very sharp and deep in the middle part of the brain and stops abruptly just caudal to the level of the foramen interventriculare. The dorsal ventricular groove appears in the 17 mm. embryo (figs. 4, 17) as a slight groove in the lateral wall some distance from the medio-dorsal angle of the ventricle.

Three sections caudal to the foramen are drawn from the 17 mm. embryo and nine sections from various levels of the 28 mm. embryo. The internal differentiation has already progressed so far in the 17 mm. embryo that it will be unnecessary to speak of it separately. In both stages many features of the sections can readily be compared with the structures seen in corresponding sections of the adult brain. For this purpose the reader should consult the description of the cell masses in the brain of Cistudo in this Journal for October, 1915. The necessary description of individual sections is given in connection with the figures. Here I shall take up the relationships of the lateral margin of the pallium and the dorsal ventricular ridge.

Corpus striatum and lateral olfactory area. In the younger embryos the loosely arranged cells of the lateral olfactory centers cover the outer surface of the caudate and lentiform nuclei and form a continuous layer over the lateral surface of the hemisphere (except dorso-caudally). This is shown in figures 7 to 12, and this interpretation is given to the models. In the 28 mm. embryo there is a small area opposite the foramen in which the striatum and the crus bundle contained in it come to the surface (fig. 21) and the olfactory centers form the pyriform lobe above and the diagonal band below. This small area is painted blue in the model and appears white in figure 5. Sections show that it is the elbow of the crus, with certain large cells contained in it ('15 b, p. 405), which comes to the surface here. This is presumably due to the rapid growth of both ascending and descending fibers in the crus. The crowding thus produced leads to the shifting of the cells of the olfactory center upward and downward. This process must go much further in later development until the large striatal area is left exposed in the adult. The embryonic history thus seems to support very clearly the hypothesis ('15 b, p. 428, 429) that the exposed striatal area in the turtle had been covered at an earlier stage of evolution by the lateral olfactory area.

Relations of pyriform lobe and pallial margin. In the caudal half of the hemisphere the pyriform lobe forms a layer external to the corpus striatum and extends up to a thin edge superficial to the dorsal ventricular ridge and the margin of the pallium. This overlapping of the pallial margin by the pyriform lobe persists in the adult. At about the level of the interventricular foramen the pyriform lobe begins to extend higher on the lateral surface. At the same time thickening of the lateral border of the pallium moves dorsally, becomes crowded and folded on itself and sinks in, forming a projection into the ventricle (figs. 22 to 25). These changes take place rapidly from behind forward so that in thirty or forty sections of 10 microns the place occupied by the thick border of the pallium comes to be taken by the pyriform lobe. From this point forward the pyriform lobe forms a characteristic ridge filled with a dense layer of cells, very much as in the adult.

Origin of dorsal ventricular ridge. In the Chelydra embryo above described the dorsal part of the lateral wall is thickened by cells coming from a proliferating area in the dorso-lateral wall. The groove which bounds this thickening was identified with the middle ventricular groove of the adult. In the older embryos of Chysemys the groove is readily identified but has been very greatly deepened by further thickening of the mass just above it. Likewise the thickening of this mass has produced a groove above it, the dorsal ventricular groove. The thick mass, then, is the dorsal ventricular ridge and in earlier stages it is indistinguishable from the dorsal pallium. In the caudal part of the hemisphere of the older embryos the relations of the dorsal ventricular ridge are not essentially changed from those seen in the Chelydra embryo. Caudal to the level of the stria medullaris (figs. 18, 19) this ridge bulges into the ventricle and is covered outside and below by the thick mass of the nucleus of the lateral olfactory tract. In the caudal pole the ridge merges gradually with the general pallium caudal to the level at which the pyriform lobe ends In the rostral part of the hemisphere (figs. 22, 23) the dorsal ridge has become practically independent of the pallial thickening, very much as it is in the adult. From these embryos it is quite clear that the dorsal ventricular ridge arises as a thickening of the lateral border of the pallium and that its continued growth causes it to project into the ventricle and produces the middle and dorsal ventricular grooves. In the 28 mm. embryo the mass of cells which in the adult was called the core-nucleus of the ridge is clearly recognizable in the rostral part, back to the level of the caudal border of the foramen interventriculare.

Between the pallial thickening and the cells of the dorsal ventricular ridge appears in the sections a clear space filled by fibers. In the caudal part of the hemisphere, where the dorsal ventricular groove is already formed, this bundle of fibers is seen to hold the same position as an important bundle in the adult. In the adult many fibers from the crus enter this bundle or, more probably, pass through it on their way to the pallium. Here in the embryo the crus is not yet sufficiently developed to enable one to trace it up through the striatum to this level. The strong development at this stage of the bundle here mentioned indicates that it is composed chiefly of fibers connecting parts of the hemisphere; an association bundle, in other words.

Relations of dorsal ventricular ridge to basal structures. In the rostral part of the hemisphere the ridge is from the first clearly marked off from the striatum below by the middle groove and by a prominent cell-free zone. In later embryos this cell-free zone becomes compressed into a narrower line as seen in section and scattering cells are found in it. There is, however, always a clear boundary between the ridge and the developing lentiform nucleus. In section the cell-free zone inclines dorsad toward the lateral surface and passes above the lateral olfactory area. In the rostral part, where the pyriform lobe crowds far dorsal this limiting zone is bent into a semicircular or U-shaped line (figs. 23 to 27). When the writer first studied the adult turtle brain he found it impossible to establish a direct comparison between the lateral zona limitans of the selachian, frog and other simpler vertebrates, and either of the cell-free zones seen in the lateral wall of the turtle brain. It is now evident that the clear zone which in the adult brain passes from a point near the middle ventricular groove toward the lateral surface and then bends dorsad to reach the surface in the sulcus rhinalis above the lobus pyriformis, is the zona limitans of the embryo and corresponds to the zona limitans lateralis in the selachian and frog. (See Johnston, '15 b, figs. 19 to 22 and compare with '11 a, fig. 75). Since the relations of the pyriform lobe, corpus striatum and pallium in the turtle are readily comparable with those in the mammal, the identification of the lateral zona limitans in the turtle completes the history of this important landmark throughout the series of vertebrates. The boundary lines between the pallial and basal portions of the hemisphere are now clear in both medial and lateral walls in fishes, amphibians, reptiles and mammals (Johnston, '11 a, '13 b, '15 b).

The relation of the dorsal ventricular ridge to the amygdaloid complex requires further comparative study. In the adult, it will be remembered, the caudal part of this ridge is separated from the rest by a broad shallow groove which continues in the general direction of the deep middle ventricular groove ('15 b, figs. 4 and 10). This caudal portion of the ridge is made up of the medial amygdaloid nucleus and of an apparent infolding of the general pallium. Even in the oldest of these embryos the dorsal ventricular ridge extends into the tip of the caudal pole as a simple thickening of the general pallium. It seems probable, although the evidence is not sufficient for a definite conclusion, that the caudal portion of the ridge including the large-celled medial amygdaloid nucleus is formed from this thickening of general pallium.

For the photographs of the models I am indebted to Dr. W. F. Allen of the University of Oregon.

LITERATURE CITED

JOHNSTON, J. B. 1911 a The telencephalon of selachians. Jour. Comp. Neur., vol. 21.
1913 b The morphology of the septum, hippocampus, and pallial commissures in reptiles and mammals. Jour. Comp. Neur., vol. 23.
1915 b The cell masses in the forebrain of the turtle, Cistudo carolina. Jour. Comp. Neur., vol. 25.

FIGURES

ABBREVIATIONS

a.p., area parolfactoria
b.o., bulbus olfactorius
c.a., commissura anterior
c.h., commissura hippocampi
ch. op., chiasma opticum
c.p.a., commissura pallii anterior
crus, crus cerebri or lateral forebrain bundle
c.st., corpus striatum
d.b., diagonal band of Broca
d.v.r., dorsal ventricular ridge
fi., fimbria
f.o., formatio olfactoria
f.p., fissura prima
for.i., foramen interventriculare
f.rh., fissura rhinalis
g.p., general pallium
g.s., gyrus subcallosus
h., hippocampus
hy., hypothalamus
l. pyr., lobus pyriformis
l.t., lamina terminalis
m.fb.bdl., medial forebrain bundle
n.c., nucleus caudatus
n.d.b., nucleus of the diagonal band
n.l., nucleus lentiformis
n.o., nervus olfactorius
n.rot., nucleus rotundus
n.tr.olf.lat., nucleus of the lateral olfactory tract
pa., pallium
pa.th., pallial thickening
p.h., primordium hippocampi
p.o., pedunculus olfactorius
r.p., recessus praeopticus
r.s., recessus superior
s.f-d., sulcus fimbrio-dentatus
s.m., stria medullaris
s.v.d., dorsal ventricular sulcus
s.v.m., middle ventricular sulcus
s.v.v., ventral ventricular sulcus
thal., thalamus
t.o., tuberculum olfactorium
tr.olf-hy., tractus olfacto-hypothalamicus
tr.op., tractus opticus

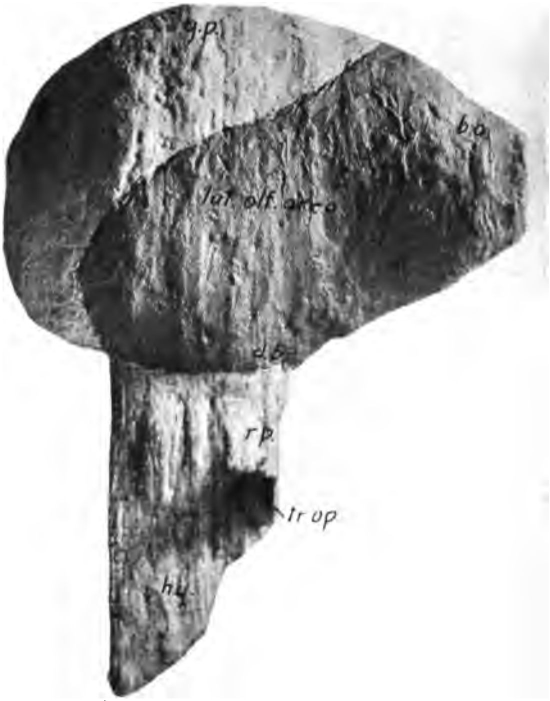

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Fig. 1 Chelydra serpentina. The size of this embryo is indicated by the owner by the words '8.5 mm. carapace.' Lateral view of a model of the right hemisphere. Description in the text.

This and the following models have been merely rubbed with a blunt instrument to smooth the surface. The external surface has been painted so that the olfactory centers and hippocampus appear dark and the general pallium light. The diencephalon has been left with the gray color of the modelling paper and the ventricular surface of the hemisphere has had no treatment whatever. The lamina terminalis and line of attachment of the choroid plexus have been painted white. All the models were made at a magnification of 50 diameters and are reduced one-third in reproduction.

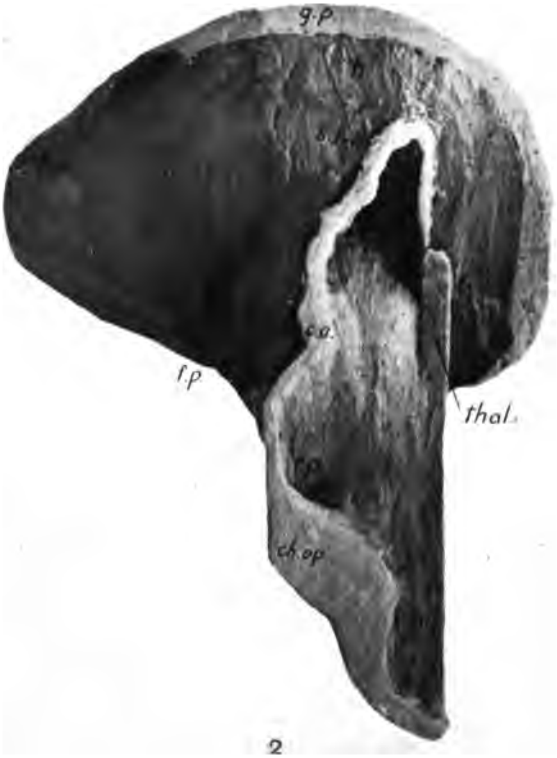

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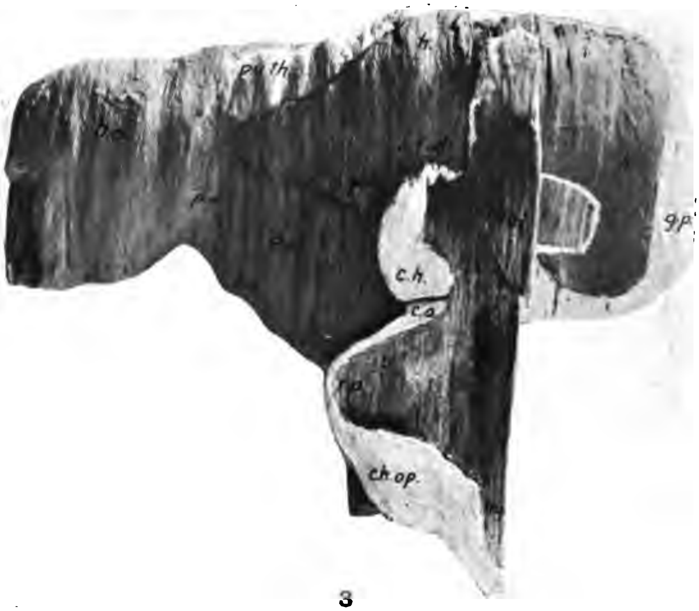
Fig. 2 Medial view of the model shown in figure 1. Description in the text.



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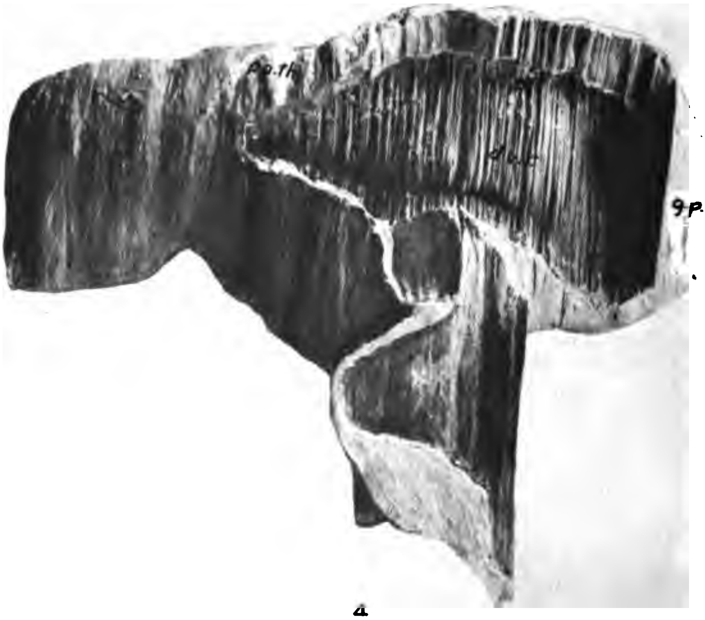

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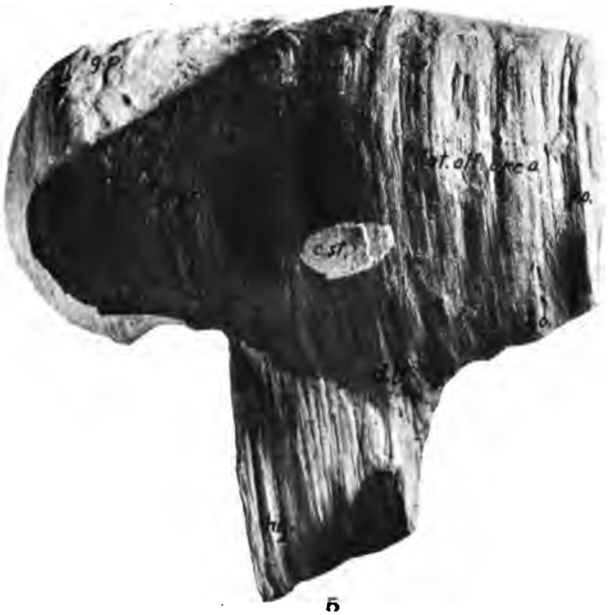

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Fig. 3 Chrysemys embryo of 17 mm. Medial view of a model of the right hemisphere. The narrowing of the interventricular foramen seems to have taken place from above downward on account of the expansion of the hippocampus and general pallium. The white area labelled *c.h.*, is the cut surface of the primordium hippocampi in the median plane. The thin portion of the medial hemisphere wall which will form the choroid fissure and plexus is bounded by a white line.

Fig. 4 Same model as figure 3, with the thalamus, hippocampus and primordium hippocampi removed. The lateral wall of the ventricle shows the dorsal ridge bounded below by the deep middle ventricular groove as described in the text. The dorsal groove is shallow. There is no evident boundary between the dorsal ridge and the pallial thickening at the rostral end.

Fig. 5 Chrysemys, 28 mm. Lateral view of a model of the right hemisphere. Note the great elongation of the pyriform lobe in the caudal pole as compared with the condition in figure 1. With regard to the exposure of the corpus striatum on the lateral surface, compare figures 1 and 5 with the figures in '15 b.

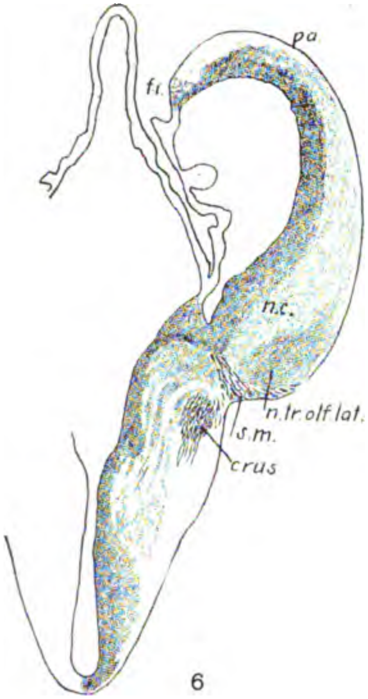
Figs. 6 to 14 Transverse sections through the right hemisphere of the Chelydra embryo illustrated in figures 1 and 2.

Fig. 6 Section just caudal to the foramen interventriculare. This is the most caudal section in which the nucleus caudatus can be recognized and here it practically fuses with the nucleus of the lateral olfactory tract. Both are undoubtedly parts of the primitive basal olfactory centers which have been carried out into the caudal pole of the hemisphere evagination. There is in this section no apparent boundary between these nuclei which enter into the amygdaloid complex and the general pallium.

Fig. 7 Section through the caudal part of the foramen, 120 microns rostral to figure 6. The nucleus caudatus is much more distinct and the proliferation of cells for the nucleus lentiformis begins to appear. There is now a slight indication of the lateral boundary of the pallium. The letters *s.v.m.* in this and figure 8 indicate the position in which the middle ventricular sulcus is to be formed, although it is not actually present in these sections.

Fig. 8 Section through rostral part of the foramen, 100 microns rostral to figure 7. The lateral olfactory area is represented chiefly by cell masses adjacent to the diagonal band. The nucleus caudatus begins to be separated from this area by the crus entering the hemisphere. The lentiform proliferation is larger and the clear space just above it, which corresponds to the zona limitans lateralis in lower vertebrates, is present from this level forward.

Fig. 9 Section through the rostral wall of the foramen. The position of the foramen is represented by the light space at the medio-ventral angle of the ventricle. The crus has now separated the nucleus caudatus from the nucleus of the diagonal band and both are somewhat removed from the larger mass of the lateral olfactory area which is here labeled *l.pyr.* The middle ventricular sulcus which is present in this section extends farther caudally in order embryos and adult.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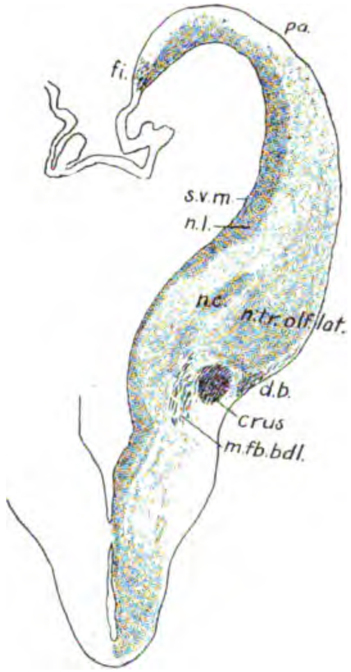
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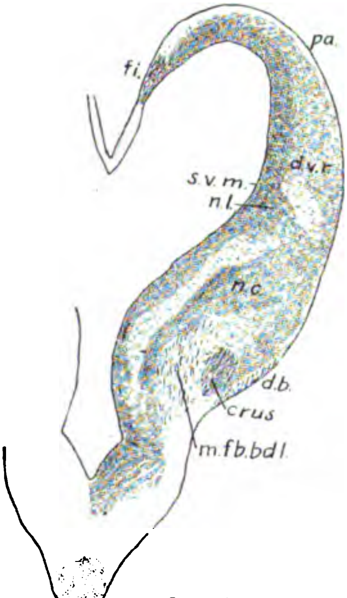
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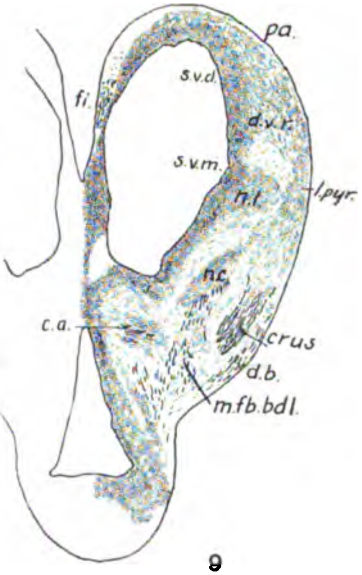
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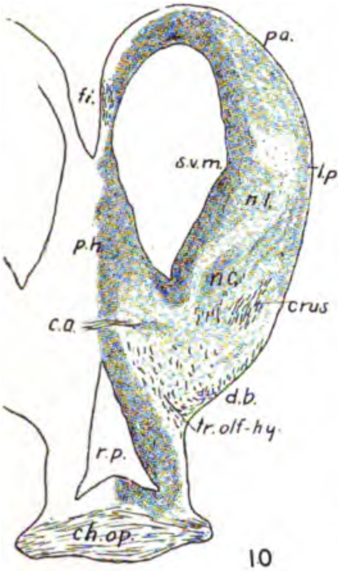
Fig. 10 Section through the anterior commissure. Although the lateral olfactory area is continuous in these sections and even the nucleus caudatus is nowhere wholly separated from it, one can clearly see the beginnings of the segregation of the pyriform lobe, diagonal band and caudate nucleus by reason of the enlargement of the lateral forebrain bundle (*crus*). This process will be completed in later stages by the development of the lentiform nucleus and the further enlargement of the crus.

Fig. 11 Section just rostral to the preoptic recess where the diagonal band turns up in the medial wall. The dark mass on the medial surface of the area parolfactoria represents the beginning of the nucleus of the diagonal band, which corresponds to the gyrus subcallosus of the mammalian brain. The nucleus caudatus is connected beneath the ventricle with the area parolfactoria. From this level rostrad the olfactory area or lobe is quite continuous from its lateral border at *l.pyr.* to its medial border near *fi.*

Fig. 12 Section 60 microns rostral to figure 11, where the diagonal band fibers rise up in the medial wall to join the fimbria. Although the cells of the pyriform lobe and pallium lie immediately in contact, there appears in many sections a very sharp line as if it were caused by a delicate septum running dorsad from the small fiber bundle beneath *l.pyr.*

Fig. 13 Section through the tuberculum olfactorium. Note that the ventral ventricular sulcus here descends nearer to the surface than elsewhere so that the somewhat bulging tuberculum contains a large pouch of the ventricle.

Fig. 14 Section through the olfactory peduncle. The ventral part of the section cuts the rostral wall of the tuberculum while its dorsal part cuts the olfactory formation.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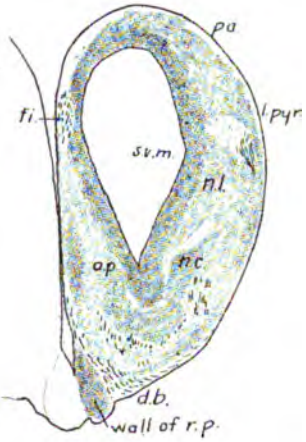
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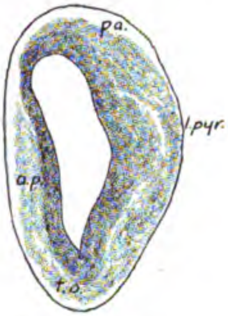
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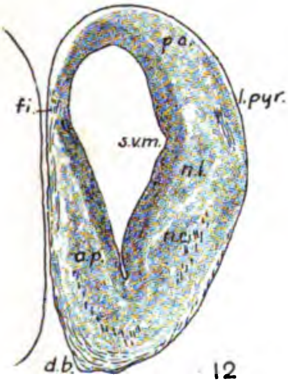
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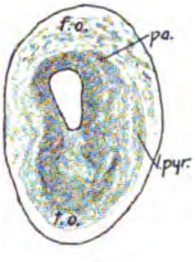
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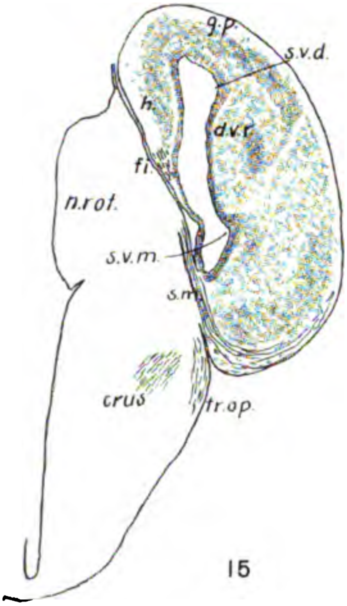
Figs. 15, 16, 17 Three transverse sections of the right hemisphere of the 17 mm. Chrysemys. Figure 17 falls in the caudal part of the foramen and figure 15 represents next to the last section in which the connection of the hemisphere with the thalamus is seen. Figure 16 is between these two.

In this embryo the mass formed by the pallial proliferation of the earlier stage has become divided into a general pallium and the dorsal ventricular ridge. The ridge projects into the ventricle forming a deep middle ventricular sulcus and a shallow dorsal sulcus. Note that the dorsal sulcus is quite independent of the dorso-medial angle of the ventricle. The dorsal ventricular ridge therefore is clearly a thickening of the lateral wall above the lateral zona limitans. This zona limitans is the light streak in the drawings running diagonally between *d.v.r.* above and *n.l.* and *l.pyr.* below. The nucleus of the diagonal band is very large and definite in figure 17, while in figure 15 and 16 its substance is fused with the nucleus of the lateral olfactory tract. The lentiform proliferation is still in progress, especially from the dorsal lip of the middle sulcus. The peculiar arrangement of ependyma cells seen at this point in the adult already begins to be apparent here.

Figs. 18 to 27 Transverse sections of the right hemisphere of the 28 mm. embryo of Chrysemys.

Fig. 18 Section at about the point where the fimbria curves down behind the choroid area. Caudal to this the nucleus of the lateral olfactory tract soon disappears and the dorsal ridge continues on into the caudal pole as a simple thickening of the lateral walls. At *g.p.?* is a partly segregated mass of cells which probably represents general pallium extending from the caudal pole forward to this point in the basal wall as it does in the adult.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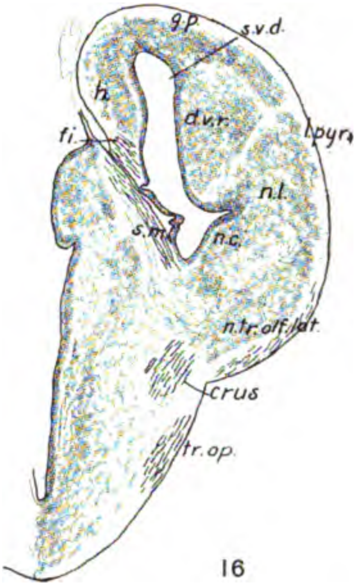
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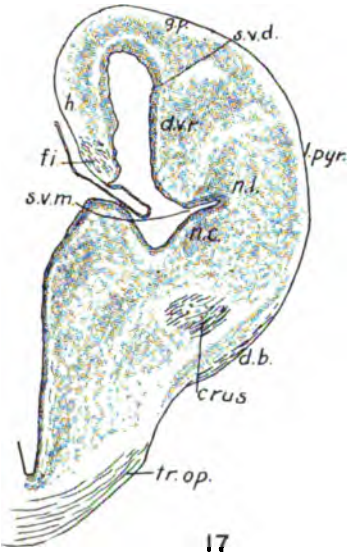
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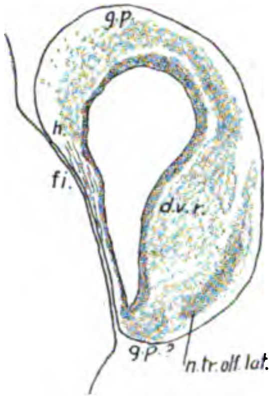
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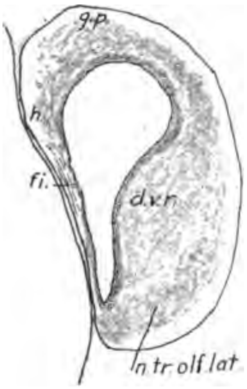
Fig. 19 Section 120 microns rostral to figure 18. In both these sections the connection of the dorsal ridge with the pallium is clear and they illustrate the more embryonic conditions which prevail in the caudal pole than in the rostral part of the hemisphere.

Fig. 20 Section at the point of connection of the hemisphere with the thalamus where the stria medullaris passes into the thalamus. In this and the next two figures the lateral zona limitans appears as a diagonal light line running dorsolaterally from *s.v.m.* to the outer surface.

Fig. 21 Section through the foramen interventriculare. The lentiform and caudate nuclei, neither of which was distinguishable in figure 20, are here quite distinct. The crus in this figure separates the caudate from the nucleus of the diagonal band. From this point rostrad for thirty to forty sections the crus lies practically exposed on the lateral surface. This condition is represented by a small striatal area in the model of this brain (fig. 5).

Fig. 22 Section through the anterior commissure. Here the general pallium begins to be decidedly thickened. The motor area of the adult includes this region and extends farther caudad. Forward from this level the olfactory area is again continuous on the lateral, basal and medial surfaces.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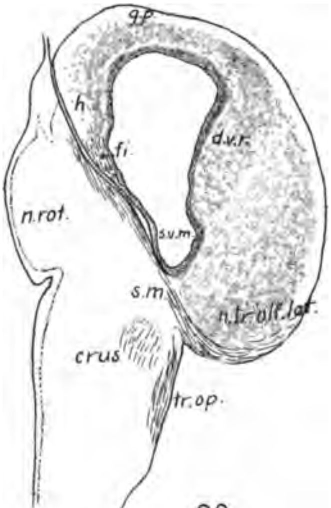
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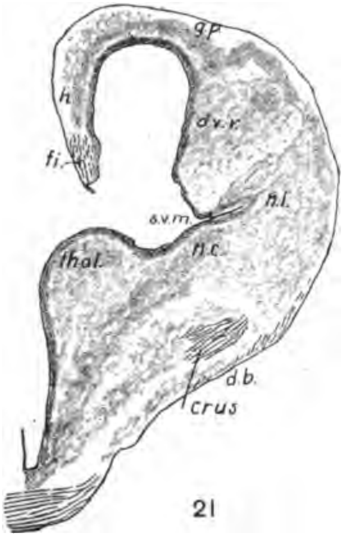
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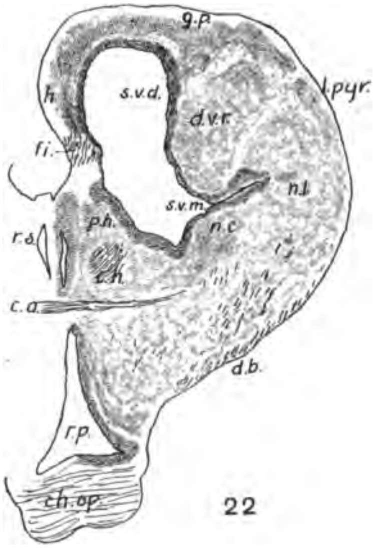
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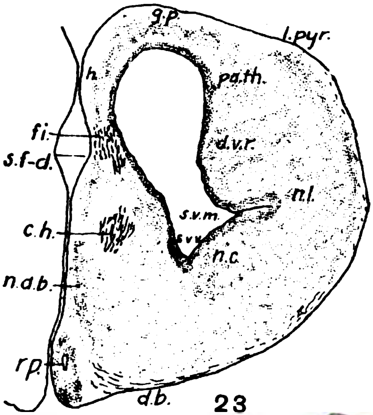
Fig. 23 Section through the rostral wall of the preoptic recess and the nucleus of the diagonal band (gyrus subcallosus). Between the level of figure 22 and this the pyriform lobe has rapidly pushed up over the outer surface of the pallial thickening as described in the text. The dorsal ridge is decreasing in size and the arrangement of its cells in this and in figure 22 suggests the core-nucleus of the adult. The dorsal ridge and the pallial thickening form a common large ridge, while in the adult they are separated by a groove (see '15 b, fig. 10).

Fig. 24 Section through the rostral end of the dorsal ridge and through the fissura prima, in which the fibers of the diagonal band are turning up into the medial wall. Both pallial thickening and pyriform lobe are massive.

Figs. 25, 26 and 27 Sections through the anterior part or motor area of the general pallium. The whole of what is called general pallium here becomes more massive in the adult and is included in the pallial thickening. An important feature of these sections is that the general pallium especially at the dorso-medial angle is composed of conspicuously large cells. If the results of experiments reported elsewhere in this journal are to be credited, this is certainly a part of the motor area and probably is concerned with the control of the limbs. The presence of the large cells is readily understood if this is true.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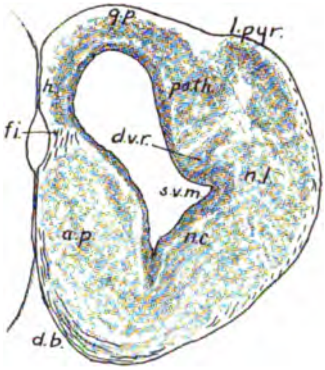
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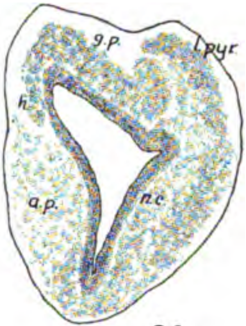
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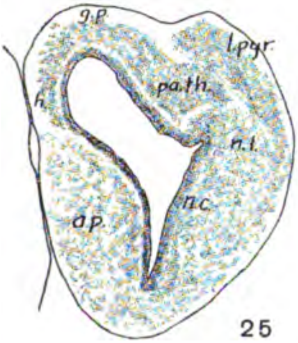
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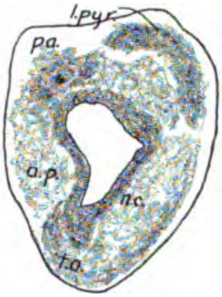
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December, 1916

NEUTRAL RED AS A CELL STAIN FOR THE CENTRAL NERVOUS SYSTEM

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The method described below has been used for eight years for the study of the tigroid content of cells as well as the size, form and grouping of cell bodies. As a 'Nissl method' it gives better results with formalin material than methylene blue or toluidin blue. The method is simple and the stain is permanent and is suitable for photography.

The stain is made up in a 1 per cent aqueous solution and kept for months or years until thoroughly ripened. The ripening process is hastened by exposure to the air or by boiling but I know of no way to obtain a satisfactory staining solution in a few days. A good stain can be had after a few months; a better one after three or four years. A 1 per cent solution is diluted as required and the dilute stain may be used over and over again.

The stain is used with either celloidin or paraffin sections. Differentiation is carried out in alcohol. The lower grades of alcohol differentiate slowly enough to allow one to examine the sections under the microscope. The higher grades wash out the stain and the sections should remain in these grades only as long as necessary to secure dehydration. If differentiation has been completed in 70 per cent alcohol, it may be carried too far while the sections are being dehydrated in the higher grades.

The clearing of the sections requires care, as any alcohol left in the sections will remove the stain. Also, some of the common clearing agents injure the stain while others soften celloidin. For paraffin sections xylol is safe and satisfactory. For celloidin sections castor oil and bergamot oil are the only common clearing agents which have proved constantly reliable. Carbol-xylol ruins the stain at once and cedar oil, clove oil, cajepat oil, oil of thyme, and anilin oil have all been unsatisfactory for one reason or another, at least in certain samples. After clearing in bergamot or castor oil it is well to rinse the sections in xylol. The mounts harden more rapidly and seem a little more brilliant and transparent. If castor oil is used the sections can be handled much more easily if the oil is thinned by addition of one part of xylol to two or three of castor oil. The xylol also counteracts any tendency of the castor oil to soften the celloidin. It is most convenient in handling large sections to float them on glass slides in the last grade of alcohol and pass them through the clearing agent on the slide. In all these particulars the method is more difficult with large and, especially, thick sections. Sections 50 microns in thickness through the whole brain of a newborn babe have been perfectly stained by this method, while sections 100 microns thick through the entire adult human brain could not be differentiated and cleared uniformly.

The steps in the process may be stated as follows:

Material fixed in formalin or alcohol. Old formalin material gives good results.
Paraffin or celloidin sections.
Aqueous neutral red Grübler (well ripened) diluted to one-fourth or one-tenth of 1 per cent.
Use warm 10 to 30 minutes, or cold 12 to 24 hours. Differentiate in 50 or 70 per cent alcohol. Dehydrate rapidly in higher grades.
Clear paraffin sections in xylol; celloidin sections in castor-xylol or bergamot oil.
Mount in dammar or balsam. Clearing and mounting media must be neutral.

Neutral red does not work well after chromic salts and hence can not be used as a secondary stain after Weigert. The stain does work admirably, however, after either the Cajal or Bielschowsky process on formalin or alcohol material. Some beautiful preparations have been obtained by treating with neutral red as above sections in which the silver stain has attacked chiefly the fibers. This enables one to study the relations of cell masses to fiber bundles and to see the origin of a silver-coated axone from the cell-body stained by neutral red.

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May, 1916

STUDIES ON THE MAMMARY GLAND

I. THE GROWTH AND DISTRIBUTION OF THE MILK-DUCTS AND THE DEVELOPMENT OF THE NIPPLE IN THE ALBINO RAT FROM BIRTH TO TEN WEEKS OF AGE

J. A. MYERS

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SIX TEXT FIGURES AND FOUR PLATES

The mammary gland undergoes many important changes from birth to old age. Numerous details in the normal structure of the gland at various stages of its history are still imperfectly known. Because of the lack of anatomical knowledge, pathologists are often unable to determine whether a certain condition of the mammary gland is due to a physiological or a pathological change. Moreover, a knowledge of the normal course of development is necessary as a basis for various lines of experimental work upon the mammary gland. In view of these facts further investigation of the developmental changes in the mammary gland during its life history seems desirable. Hence a series of studies upon this subject has been undertaken. The present paper, which is the first of the series, deals only with the growth and gross relations of the ducts, and the gross development of the nipples from the first day to ten weeks after birth In later papers, the prenatal condition as well as various changes involved during pregnancy, lactation, and involution will be considered.

MATERIAL AND TECHNIQUE

The present study is confined to the mammary gland of the albino rat (Mus norvegicus albinus). This form is easily reared in the laboratory and is thus available at all times. Its life cycle is short, so that any desired stage in the history of the mammary gland can be obtained in a comparatively short time. The various developmental stages are therefore easily obtained and controlled.

In the present study three methods were used. (1) Microscopic sections were studied; (2) wax reconstructions (of the newborn) were made according to Born's method; (3) the ducts were studied from cleared preparations. These preparations were made according to the method employed by Lane-Claypon and Starling ('06). The skin of the entire ventral part of the body was removed, spread out on a sheet of cork, and fixed in a mercuric chloride-formalin solution (10 per cent formalin in a saturated aqueous solution of mercuric chloride). The corium and tela subcutanea containing the gland were then removed in a single sheet. In the older specimens it was usually necessary to dissolve out the fat with alcohol and ether before staining. The preparations were then placed in a very dilute solution of alum-hematoxylin or carmalum until they were sufficiently stained. When necessary, the excess stain was washed out with acid-alcohol. After dehydration, beechwood creosote and cedar oil were used as clearing agents, the specimens being first placed in creosote for a few hours and then transferred to cedar oil. After being thoroughly cleared they were mounted in damar on glass slides.

Frank and Unger ('11) state that Starling's technique of staining and clearing the breasts, as in the rabbit, could not be employed in the rat. No explanation is given as to why Starling's method could not be employed. It is true that this method is rendered quite difficult owing to the development of the panniculus carnosus muscle in the thoracic region. Being very closely related to the milk-ducts, it is almost impossible to dissect this muscle off without destroying some of the mammary gland. However, good cleared preparations can be studied to advantage even when the muscle is intact. The fact that the panniculus carnosus muscle is lacking in the abdominal and inguinal regions makes the study of the glands in these regions comparatively easy. Fat is quite easily removed in cleared preparations, hence the considerable quantities of it deposited in the region of the abdominal and inguinal glands do not add many difficulties in the technique.

Sections for microscopic study were prepared by fixation in Zenker's fluid, and embedding in paraffin. The sections were cut 10 micra thick, mounted serially and stained with Mallory's connective tissue stain or with alum-hematoxylin.

The albino rats used were in good health. After weaning, at the age of three weeks, they were fed upon whole wheat (Graham) bread, soaked in whole milk. They were in general of average weight or above, as indicated by the following gross body weights of the rats from which the specimens represented in the figures were obtained: newborn, about 4.5 grams; 1 week, 8.5 grams; 2 weeks, 15.5 grams; 3 weeks, 30 grams; 4 weeks, 53 grams; 5 weeks, 54 grams; 7 weeks, 75 grams; 9 weeks, 114.5 grams.

OBSERVATIONS

1. General arrangement of glands and ducts

As shown in figure 1, the mammary glands of the albino rat may be grouped according to the regions which they occupy. The three most cephalic pairs of glands lie in the thoracic region, hence are designated thoracic mammary glands. Passing caudad, the next pair lies in the abdominal region at about the level of the umbilicus. Henneberg ('00) speaks of this pair as the abdominal mammary glands. The two remaining pairs, known as the inguinal mammary glands, lie in the inguinal region.

Each of the first pair of thoracic glands lies slightly cephalad to, or at the level of, the fore limb. Each adult nipple is located about 10 to 12 mm. from the mid-line. In the case of the second pair of thoracic gland as a nipple is found immediately behind each forelimb. Here the distance from the nipple to the mid-line is somewhat greater, being about 15 to 16 mm. The distance between this and the first pair of thoracic glands is normally about 30 mm. The last pair of thoracic glands is very closely associated with the second pair, the nipples being only about 12 to 15 mm. apart. The distance from each nipple to the mid-line is approximately 35 mm. The abdominal pair of glands stands somewhat isolated from the others, lying about 50 mm. caudad to the last thoracic and 25 mm. cephalad to the first inguinal. As stated above, these glands lie near the level of the umbilicus. Their distance from the mid-line is about the same as, or slightly greater than, that of the last thoracic glands. Each nipple of the first pairs of the inguinal glands lies in the inguinal region immediately medial to the thigh. The distance from the nipple to the mid-line is approximately 12.5 mm. The nipples of the second pair of inguinal glands lie in the most caudal part of the inguinal region. They are located latero-cephalad to the urethral orifice. Like the first thoracic glands, the nipples of the last inguinal glands approach the mid-line, the distance being only about 10 to 12 mm. It should be observed that in passing caudad from the first thoracic glands the distance from the nipples to the mid-line gradually increases until the last

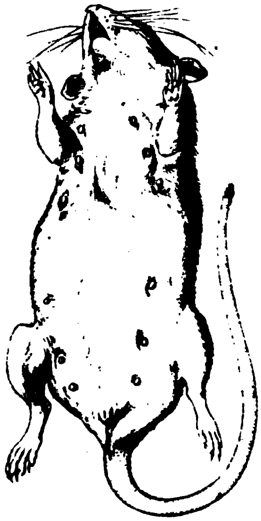


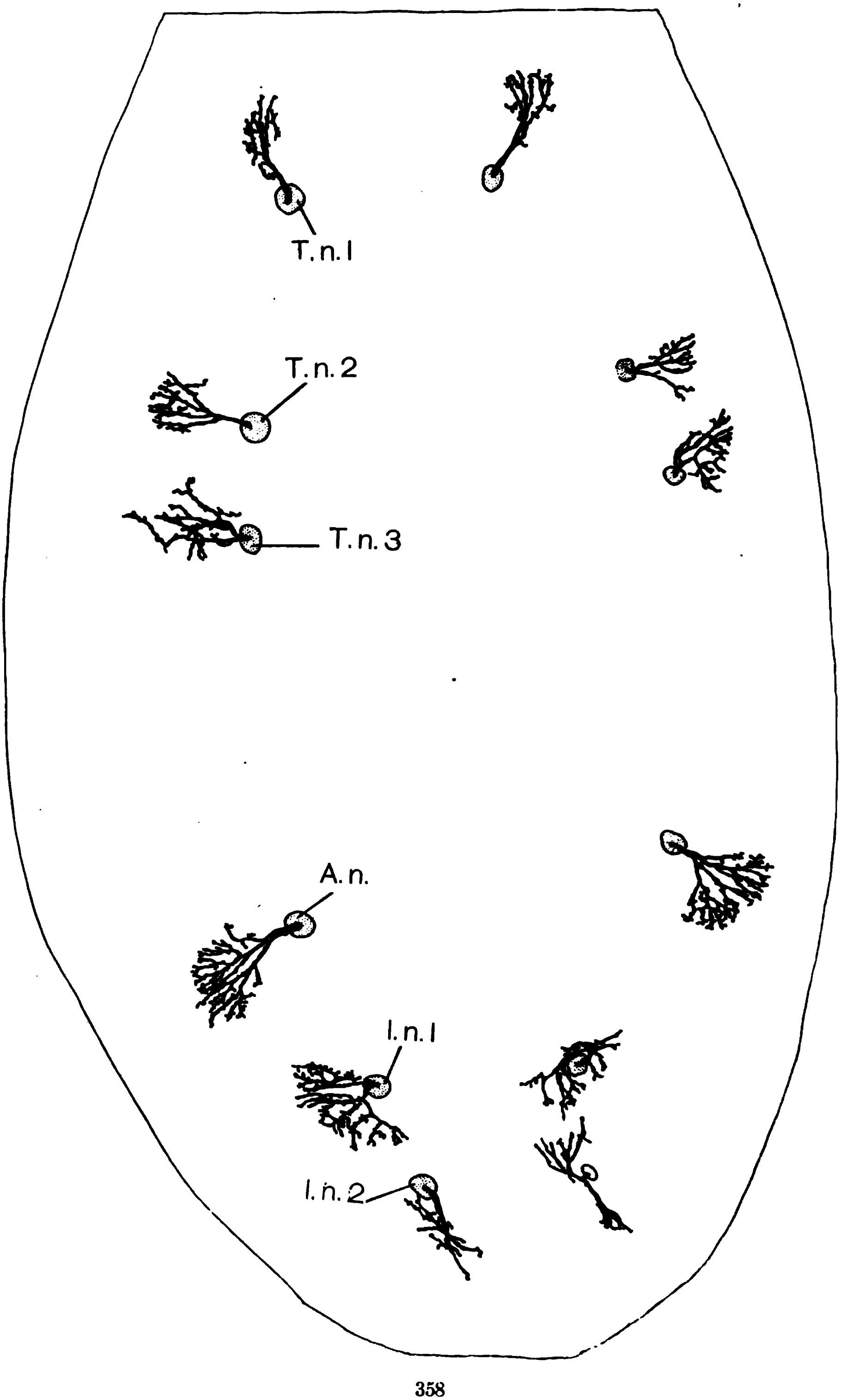
Fig. 1 Ventral aspect of adult female rat during lactation, to show location and arrangement of the nipples (from Henneberg '00).

thoracic pair is reached, remains about the same for the abdominal pair, then decreases to the last inguinal pair.

From figure 1 and the above measurements it will be observed that the abdominal pair of glands is more closely associated with the inguinal than with the thoracic glands. This arrangement points toward a localization of the glands in only two regions. So far as the distribution of the mammary glands is concerned, the rat may be regarded as occupying a position between those forms which possess a continuous row of mammary glands on each side from the thoracic through the inguinal regions and those in which the mammary gland is confined to a single region.

The average number of mammary glands in the albino rat is 12 (6 pairs), but Henneberg ('00) and Frank and Unger ('11) have called attention to the fact that the number varies between 10 and 14. Henneberg examined 28 embryos from the age of 14 days and 20 hours to 15 days and found in 5 cases that supernumerary mammary hillocks appeared. All were in the pectoral region. The accessory hillocks, which in all cases appeared smaller than the normal, were located in 4 cases between the second and third normal hillock, and in one case caudal to the third. In the adult animals, however, he found that hypermastia rarely occurred, as only one case was reported from 150 observations. In this one case the individual possessed a pair of apparently functional glands just caudad to the last pair of thoracic glands. In the 150 adult rats only one case of hypomastia was found. In this case the individual lacked the first pair of inguinal glands.

In the present study, the number of glands present was noted in all animals available. From observations made on 100 individuals ranging in age from 10 days to adults, 80 were found to possess the normal number of mammary glands. Only one supernumerary gland was observed. It was located just caudad to the third thoracic gland on the left side, the right side presenting the normal number. In 12 cases the second thoracic gland was apparently lacking on the right side only, while in 7 other cases the second thoracic gland was lacking on both sides.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A very good time to make these observations on the albino rat is about the tenth to the fourteenth day of life. As pointed out by Jackson ('12), the mammary glands (nipples) are very conspicuous at this time. After the first two weeks have passed, it is very difficult to make accurate observations as the glands are well covered with dense hair. During pregnancy and the period of lactation the glands again become very conspicuous.

Just medial to the apex of each nipple is a single opening which leads into one large duct. This duct after reaching the tela subcutanea turns almost at right angles and courses through this layer parallel to the surface. Instead of receiving a large number of tributaries from all directions, this single duct at first receives only a small number of tributaries usually from a single direction. Figure 2, drawn from a cleared preparation of the integument of a rat two weeks old, will serve to show the general direction taken by the ducts of each gland. Here it may be observed that the main duct of the first thoracic gland extends cephalad, then breaks up into numerous branches. No ducts are seen to pass out in any other direction from the nipple. The general direction of the ducts of the second thoracic gland is somewhat different as they pass almost directly laterad from the nipple. In a number of cases, however, these ducts were found to extend somewhat latero-cephalad. A larger number of branches lead from the third duct than from either of the other thoracic nipples. The ducts of the third take the same direction as do those of the second thoracic gland. The abdominal gland, whose duct shows a greater amount of branching than any of the others, sends its branches in a caudo-lateral direction. The main duct of the first inguinal gland, after passing a short distance caudo-laterad, breaks up into ramification some branches of which take a cephalic while others take a caudal direction. The duct of the last or second inguinal gland usually sends all of its branches directly caudad. In some specimens, however, a few branches

Fig. 2 Drawn from a cleared preparation of a two weeks' albino rat (internal view) to show the general arrangement of the nipples and the branching of the mammary ducts. × 4. *T.n.1*, *T.n.2*, *T.n.3*, 1st, 2d and 3d thoracic nipples; *A.n.1*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples.

pass cephalad. It is evident from the drawing of the surface of the abdomen (fig. 1) that the nipples are arranged on each side so as to form somewhat of an arch. It should be noticed in figure 2 that the arch effect is also carried out by the direction and distribution of the ducts.

The milk-ducts of late human and rabbit fetuses were divided by Rein ('82) into several subdivisions as follows: (1) the inflated terminal branches of the 1st, 2d, and 3d orders which together form the outline of the secretory part of the gland; (2) that part extending from these ramifications to the mammillary zone which represents the future lactiferous sinus; (3) the intramammillary portion which constitutes the proper excretory duct; (4) the infundibuliform part which passes through the epidermis. Brouha ('05) was unable to adopt the subdivisions proposed by Rein, because neither at birth nor even in the course of the first month of postnatal life was he able to distinguish any part which was differentiated into a lactiferous sinus. Owing to this fact Brouha suggested and followed a somewhat different subdivision. He considers each milk-duct composed of three segments; (1) the intra-epidermal infundibuliform segment; (2) the excretory segment which extends from the preceding to the place of the first bifurcation; (3) the secretory segment, which is composed of the succeeding branches. This subdivision Brouha bases upon the later histological characters of the different portions of the lactiferous arborization.

A somewhat different classification of the ducts is used in this paper. That part of the duct passing through the epidermis is called the intra-epidermal portion of the primary duct. That part extending from the epidermis to the first division is designated the primary duct. The ducts resulting from the divisions of the primary ducts are spoken of as the secondary ducts. The secondary ducts divide into the tertiary ducts. The collateral ducts are those given off from the sides of the main ducts. All those ducts which end blindly are called terminal ducts.

When the various pairs of glands are examined it will be observed that for certain features a single general description can be applied to the ducts of all. However, each varies more or less from a general type; and it thus becomes necessary in some places to make the description somewhat detailed. This description is given later under "Growth of the ducts."

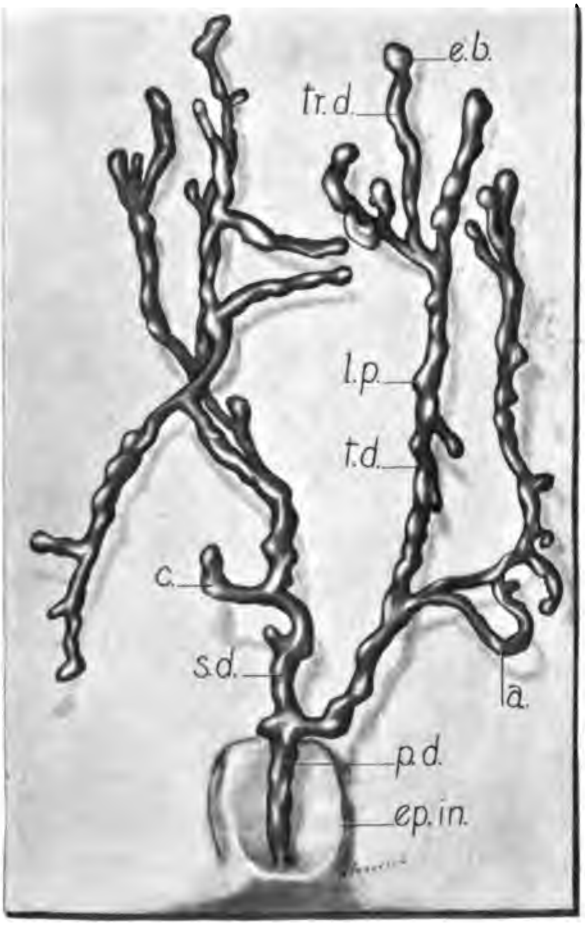

Fig. 3 Internal view of a wax model reconstructed from the left second thoracic gland of a newborn albino rat. × 40. *a*, anastomosis; *c*, collateral duct; *ep.in.*, epithelial ingrowth of nipple; *e.b.*, end-bud; *l.p.*, lateral process; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct.

In figures 3 to 6 (from wax reconstructions of the newborn) the intra-epidermal portion of the primary duct is not visible, but the primary duct is seen to emerge from the inner surface of the epidermal layer of the skin, pass deeply into the tela subcutanea and suddenly turn at right angles after which it lies paral-

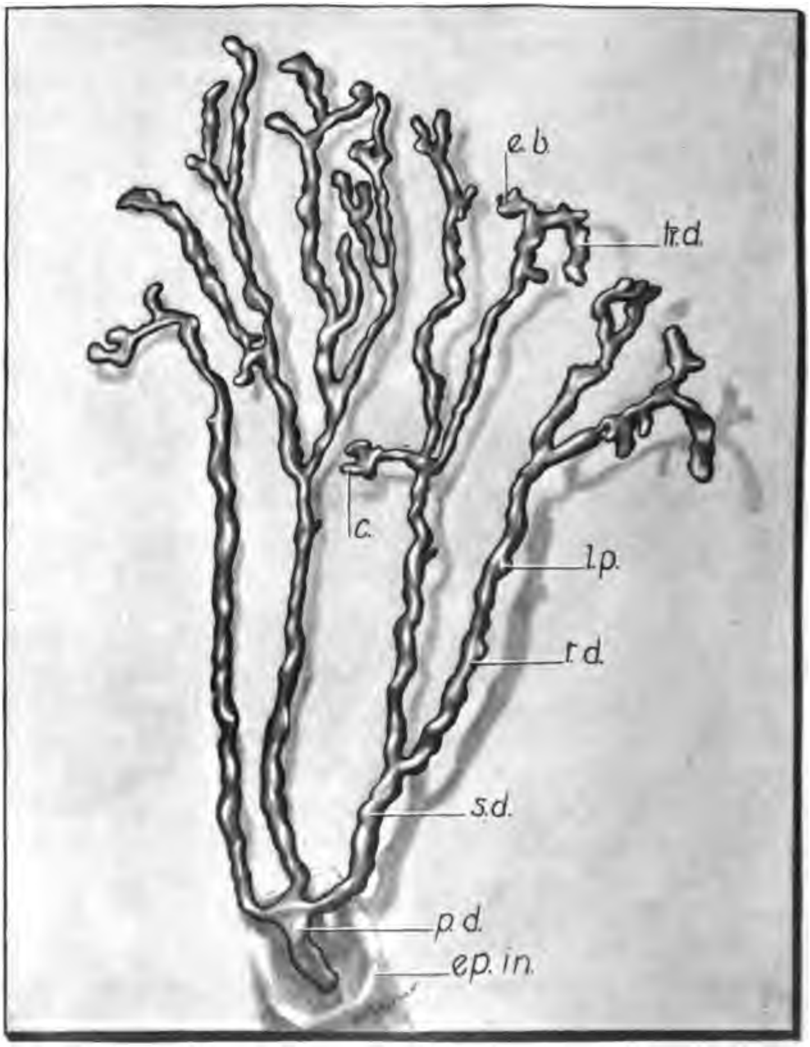

Fig. 4 Internal view of a wax model reconstructed from the right third thoracic gland of a newborn albino rat. × 40. (For lettering, see fig. 3.)

lel to the surface of the skin. In most cases observed the primary duct extends only a short distance until it divides into two branches (secondary ducts) nearly equal in size.

The extent of the primary duct varies considerably. For instance, in the first thoracic and the last inguinal glands the primary ducts present a rather extensive course before dividing, while in the remaining glands they divide almost immediately after making a sharp turn in the tela subcutanea. In figure 4 (last thoracic gland) the primary duct is seen to divide into three branches. This is an exception to the general rule that the primary duct divides into two branches.

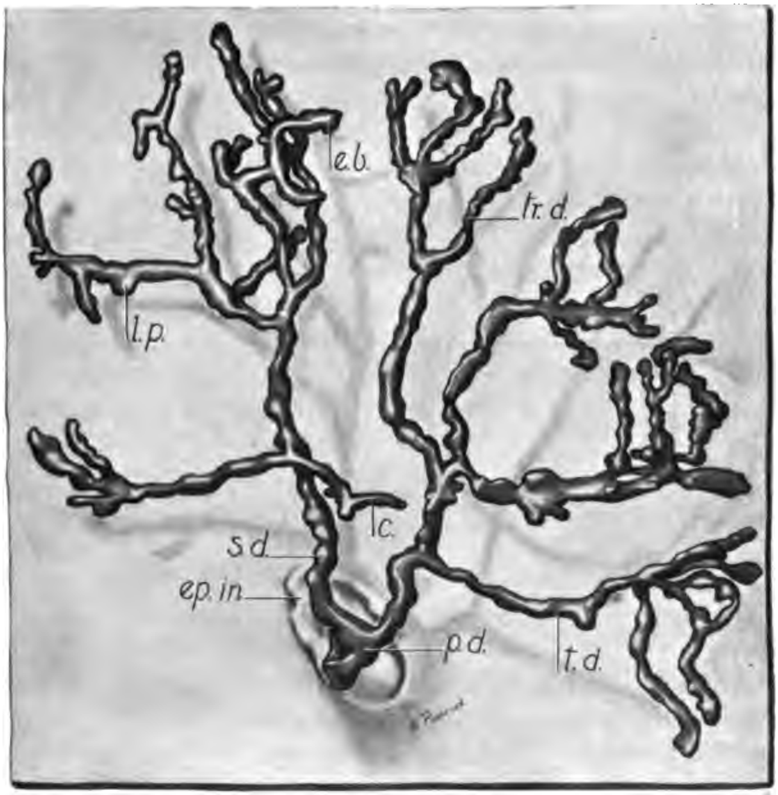

Fig. 5 Internal view of a wax model reconstructed from the left first inguinal gland of a newborn albino rat. × 40. (For lettering, see fig. 3.)

As compared with the primary duct, the secondary ducts present a rather extensive course, after which they break up each into two or more branches (tertiary ducts). It will be noticed that at birth (figs. 3 to 6) the terminal branches of each tertiary duct vary from one to three in number. On the end of most terminal branches is a small bud-like enlargement. These enlargements were described as true alveoli by earlier investigators, but this was found later to be incorrect. Billroth (according to Berka '11) doubts whether completely formed end-vesicles occur in young human virgins. While he called the terminal enlargements 'real end-vesicles,' yet he adds that they later develop into '*true* end-vesicles' and further multiply during pregnancy. Berka ('11) states that true alveoli donot occur in young (human) virgins. Similarly the terminal enlargements found on the milk-ducts of young rats are not true alveoli, but are only enlarged growing processes corresponding to the end-buds found in other developing glands. The microscopic structure of these enlargements and the development of true alveoli will be discussed in a later paper dealing with the histology of the mammary gland.

The question often arises as to whether the ducts of glands branch dichotomously or otherwise. From the various figures it will be seen that the more proximal parts of the terminal segments usually follow the dichotomous method, but the distal portions, as stated above, may terminate as a single duct or divide into two or three branches. In the last thoracic gland (fig. 4) the secondary branches approach true dichotomous division.

Anastomoses occur between ducts, but they are not very frequent in the newborn rat. In the reconstructions made from glands at birth, only two distinct anastomoses occur (fig. 3). However, others have been observed in cleared preparation at the same stage.

It will be noted that along the secondary and tertiary ducts numerous lateral buds occur (figs. 3 to 6). Many more of them are present on the distal than on the proximal ducts. Such buds later form collateral branches destined to develop into ducts similar to those already present. This point will be more clearly brought out in the older stages.

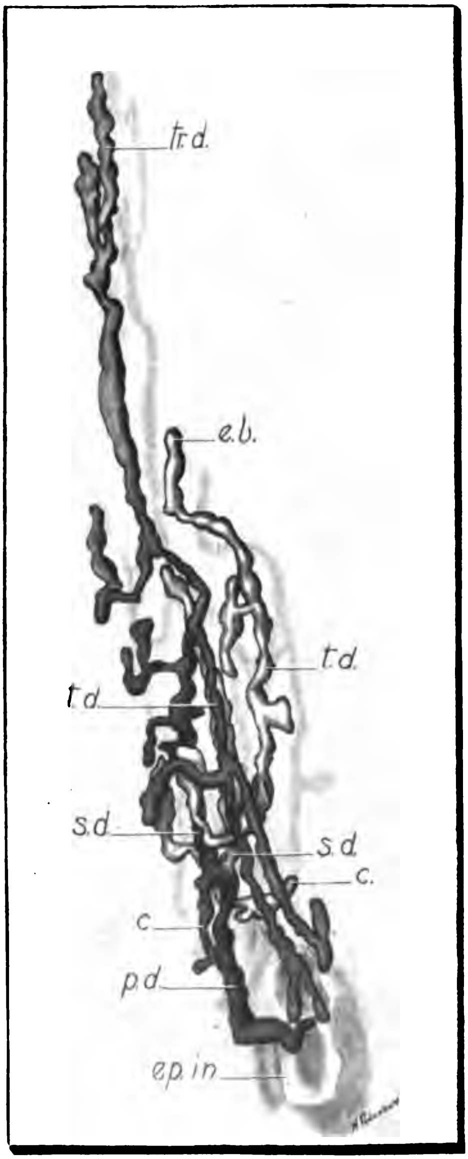

Fig. 6 Internal view of a wax model reconstructed from the left second inguinal gland of a newborn albino rat. × 40. (For lettering, see fig. 3.)

A point which has been discussed at some length recently and one which has proved to be of considerable importance in experimental work is that of the variation in the relative size and development of the various glands in the same individual, and of glands from different individuals of the same age. Lane-Claypon and Starling ('06) in working on the growth and activity of the mammary gland concluded that breast hyperplasia of pregnancy is caused by chemical substances formed in the embryo. Such substances passing through the placenta into the maternal blood-stream cause growth of the mammary gland. To decide definitely as to just what tissues cause this growth Lane-Claypon and Starling injected extracts of placenta, placenta and uterus, ovaries, fetus, fetus together with the placenta and membranes, and mucous membrane of the uterus into virgin rabbits. Some of the extracts when injected caused very little apparent change in the size of the mammary gland of virgins, while others (fetus extract, for example) seemed to cause a marked development of the glands.

Frank and Unger ('11) in repeating certain of Lane-Claypon and Starling's experiments obtained different results, and furthermore found that their own series of experiments did not show uniform results. Thus they concluded that some disturbing factor remained to be accounted for, so they decided to study more carefully the anatomy and the physiology of the normal mammary glands of the rabbit. For such study they selected a number of apparently virgin adult female rabbits and under the necessary precautions removed a mammary gland from each. At various intervals of time other mammary glands from the same animal were removed and studied. From these experiments Frank and Unger were able to demonstrate in virgin rabbits changes which were indistinguishable from those seen at the end of the first third of pregnancy. Thus some physiological factor must be involved. Frank and Unger found a partial explanation for this condition in an article by Bouin and Ancel ('09) who describe variation in the size and appearance of the rabbit's mammary gland corresponding to the development of the corpus luteum. A little later O'Donoghue ('12) showed that there is a decided change in the structure and size of the mammary glands of Dasyurus viverrinus when ovulation is not succeeded by pregnancy.

A comparison of the individual glands of the rat at birth and at two weeks (figs. 2 to 6) will show that there is considerable difference in the size and development of the various glands in the same rat, sometimes even in the same pair of glands (fig. 2). It has also been observed that corresponding glands from different rats of the same age and approximately equal weights show considerable variation in size and complexity of structure. The differences in size and development observed by me in the rat are not so marked as those described by Frank and Unger, Bouin and Ancel, and O'Donoghue. Yet they are worthy of mention and are certainly sufficient to prove that the normal structure and variability under different conditions of any part of the animal body should be thoroughly investigated before conclusions are drawn from experimental work. It is quite possible that such knowledge of the mammary gland of the rabbit would have changed decidedly the conclusions of Lane-Claypon and Starling.

2. *Growth of the ducts*

In the newborn rat, models were reconstructed showing one gland of each of the six pairs (figs. 3 to 6). At two weeks, all the glands are represented in figure 2, to show the general topography of the ducts. At the other stages (1 week, 2, 3, 4, 5, 7 and 9 weeks, figs. 7 to 13) it is found unnecessary to reproduce all the glands, so only the abdominal and inguinal glands of the left side are shown. A general description of all glands at each stage will be given, however.

Newborn. The figures drawn from wax reconstructions of the glands at birth (figs. 3 to 6) show that with few exceptions the ducts of each gland in these stages all lie approximately in the same plane, parallel to the surface of the skin. It was noticed that in regions where there are no obstructions the ducts spread very freely and cover a considerable area. The unobstructed ducts almost invariably lie in a single plane at this stage of growth. The last thoracic and the abdominal glands (fig. 4) are good examples. Here, as is readily seen, the integument is free from appendages or anything that would tend to limit the uniform spreading of the ducts. It was also noticed that in regions where there are obstructions the branching of the ducts is more irregular and that the ducts arrange themselves so as to lie in more than one plane. The best example of this condition is seen in the last inguinal mammary gland (fig. 6). Here as previously stated the nipple lies in the caudal part of the inguinal region. To the lateral side of this gland the area available for ramification of ducts is obstructed by the hind-limb while to the medial side the external urinary and genital organs limit the area. This leaves only a very narrow region free for the distribution of ducts. Consequently, instead of spreading freely and occupying a single plane, the ducts branch so as to lie in three or four planes, each of which is parallel to the surface. Small areas where the growth of the mammary gland is obstructed by lymphatic glands are shown in several of the figures of later stages. Thus the course, branching, and spreading of the ducts depend largely upon the available space.

The reconstructions show that the ducts of the abdominal and inguinal glands lie much deeper from the surface than those of the thoracic glands. This is probably due to the absence of the panniculus carnosus muscle which may to some extent prevent the ducts from passing deeply in the thoracic region. Also considerable fat is present in the abdominal and inguinal region, which is apparently a very favorable substance for the ramification of ducts.

First week. At the end of the first week the ducts are not much different from those at birth except that they are slightly more branched. The ducts of the first thoracic glands give off a few branches which take a caudal direction; all the other branches of this pair of glands pass cephalad. The second pair of glands sends the ducts in a latero-cephalic direction, but many collaterals are given off some of which take a cephalic while others take a caudal direction. The third thoracic glands send their ducts in the same general direction as the second. The greater number of the collateral ducts of this gland pass so far cephalad that only a comparatively small space exists between them and the caudal collaterals of the second thoracic gland. The caudal collaterals are few in number, yet quite long.

The ducts of the abdominal glands (fig. 7), which are most branched of all, send their branches in a latero-caudal direction. Many collaterals are given off, more of which take a caudal than a cephalic direction. In the case of the first inguinal gland, the number of collateral branches taking the cephalic direction is about equal to the number taking the caudal direction. The ducts of the last inguinal glands send the majority of their branches directly caudad; however, a few branches may be seen passing cephalad toward the first inguinal gland. Terminal end-buds are very prominent on all the glands at this stage. Also the small lateral buds are numerous but they are largely confined to the more distal ducts.

Two weeks. Figure 2 represents all of the mammary glands of a rat at the end of the second week of life. Figures 2 and 8, together with the description given in an earlier part of this paper, render further description unnecessary.

Three weeks. The three weeks' specimen from which figure 9 was drawn shows less branching than the one-week stage, but the ducts are greater in diameter. This specimen greatly emphasizes the marked variation in the development of the glands in different individuals, since in this instance the glands of an individual one week old show greater development than those of another individual three weeks old. Also, as pointed out above, the glands of one side may show more advanced stages of development than corresponding glands of the opposite side.

Four weeks. At the end of four weeks the first pair of mammary glands does not show a marked increase in development over the two-weeks stage. One very noticeable difference is that the proximal parts of the secondary ducts bear a large number of lateral buds similar to those appearing on the distal parts of the same segment of the earlier stages. Such processes are much less numerous on the corresponding parts of the second and third thoracic glands. The second glands in some of the specimens show a very slight increase in development over the two-weeks and three-weeks stages. The ducts of the third thoracic glands show a much greater development than has been observed in any of the earlier stages. The ramifications are so numerous that some are seen to pass superficially while others take a deep course. This arrangement necessarily takes them out of the plane of the main ducts. Thus from this stage the ducts become so crowded that it is impossible for them all to occupy a single plane, as found at birth in all except the last inguinal glands. Large numbers of branches from the ducts in the third thoracic gland also take a cephalic course, which is so extensive on the right side that they very nearly come in contact with the ducts of the second thoracic gland. On the left side the interval between the ducts of the two glands is greater. Figure 10 shows that the abdominal and inguinal glands present much richer arborizations than corresponding glands of earlier stages. Some lateral buds are developed on the secondary ducts yet they are not so numerous as in the first thoracic gland of the same stage. The general course taken by the ducts is the same as that given in previous descriptions. A well defined interval exists between the ducts of the abdominal and the first inguinal glands. There is also a considerable space existing between the ducts of the first and second inguinal glands. However, the second inguinal has sent numerous ramifications in the direction of the first inguinal gland.

Five weeks. During the fifth week the ducts increase very rapidly in length. Also a great many new branches spring from the more distal ducts of the glands. The first thoracic gland is much more complicated than in any of the previous stages studied. Between the ducts of this and those of the second thoracic gland a wide interval still exists. The interval between the ducts of the second and those of the third thoracic glands has largely disappeared on both sides, there being a slight overlapping of a few of the ducts from each gland. In the specimens observed, however, there are no anastomoses present between the ducts of the two glands.

During this week the abdominal and inguinal glands (fig. 11) also undergo very rapid development. This is especially noteworthy in view of the fact that the body weight of the rat from which the gland at five weeks was drawn was practically the same as that of the rat used at four weeks (fig. 10). The ducts of the abdominal and first inguinal glands at five weeks (fig. 11) interlace very intricately. With the aid of the microscope (especially the binocular) one can be reasonably sure that many of the ducts simply overlap, there being no anastomoses. However, there are areas in which it is impossible to decide definitely as to whether true anastomoses occur. This holds true in all the later stages. Further investigations are necessary to determine this point concerning anastomosis. In this same week the ducts of the second inguinal glands on each side have grown cephalad to meet, and in some places even overlap, the ducts of the first inguinal. Here the overlapping is not very complicated and one can see distinctly that no anastomoses occur. The ducts of the second inguinal gland taking a caudal direction branch very profusely.

Six weeks. At the end of the sixth week the glands do not differ greatly from the five-weeks stage. Some of the glands, the abdominal for example, show greater development. Others, as the second inguinal, reveal no increase; in fact, in some specimens they are less developed than those of the five-weeks stage. The terminations of the ducts of the first and second inguinal glands are separated from each other by considerable space.

Seven weeks. In the seventh week stage, the caudally directed ducts of the first thoracic gland have made considerable advance toward the cephalically directed ducts of the second thoracic gland. However, they are still separated by a space of five to eight millimeters in width. As in some of the previously described stages the ducts of the second and third thoracic glands overlap. The branching of the ducts of these glands is somewhat more complicated at this stage. The abdominal and inguinal glands (fig. 12) present very complicated systems of ducts; also the overlapping between the ducts of the first and second inguinal glands is very marked at this time.

Eight weeks. In the eight-weeks specimens, the first and second thoracic glands have increased to such an extent that their ducts overlap. This overlapping may occur on one side only, there being an interval between the corresponding ducts of the opposite side. Concerning the other glands of this stage, very little need be said except that the ducts have increased in length and new branches have been added. It should be pointed out that at this stage the rat possesses only four distinct masses of mammary gland tissue. All of the thoracic glands of each side have their ducts so interlaced as to form one apparently solid mass of ducts. Also the abdominal and inguinal glands of each side are so matted together that no dividing line exists between them.

Nine and ten weeks. The nine and the ten-weeks stages (see fig. 13) show a tremendous increase in development over the previously described stages. The ducts have spread out to cover a much larger area. Not only is the overlapping of ducts more complicated but each gland has produced a large number of medial and lateral branches. For example, some of the medial ducts of the first thoracic glands of each side have grown so near to the mid-line that only a narrow space separates them. In the case of the first inguinal glands the medial ducts actually reach the mid-line, and a slight overlapping of the ducts of opposite sides occurs. The medial ducts of the second inguinal glands almost surround the vagina, the ducts from the opposite sides very nearly meeting in the mid-line both cephalad and caudad to the vagina. At this stage the proximal or secondary ducts also bear large numbers of short collateral ducts which have developed from the lateral buds mentioned in the earlier stages.

It is frequently stated that from birth to puberty the human milk-ducts undergo very little development, merely keeping pace with the general body-growth. At puberty an abrupt change like that affecting the entire organism is said to occur. So far as the rat is concerned, an examination of figures 2 and 7 to 13 will show that in the virgin, from birth to the age of ten weeks, there are apparently two periods when the increase in the mammary gland ducts is somewhat marked. The first period occurs about the fourth and fifth weeks. Whether there are definite factors causing this first increase has not been determined. It is possible that it is due to individual variation. It does not appear to be due to any greater relative increase in the body weight at this period, and is of doubtful significance.

The second and more important period of increase in the mammary gland occurs about the ninth week. Donaldson ('15) states that the female rat arrives at the age of puberty about 60 to 70 days after birth, the gonads indicating sexual maturity at the age of two months or less. Jackson ('12) however, found pregnancy to occur in one case at the age of seven weeks. Lantz ('10) cites from Buckland a case where a white rat is said to have given birth to 11 young at the age of eight weeks (and which accordingly must have become pregnant at the age of five weeks). These are very exceptional cases, however.

Jackson ('12) states that the vaginal aperture does not appear until the middle or end of the second month. There is considerable individual variation on this point. The average taken from fifteen observations by me is 8.3 weeks. Therefore, the marked increase in the development of the mammary glands of the rat between the eighth and ninth weeks evidently corresponds closely with the age of puberty. Thus the second marked increase in the size of the mammary glands is readily accounted for.

3. *Lumen of the ducts*

The time of appearance and method of formation of the lumina of the milk-ducts in various animals have been desribed by various authors. In the albino rat at birth a small irregular slit-like lumen is present in the primary duct (fig. 14). This lumen when traced proximally disappears in the intra-epidermal portion of the primary duct, but when traced distally becomes continuous with the more regular rounded lumen of the secondary ducts. The tertiary and in fact all of the remaining ducts at birth possess lumina throughout their entire extent. The terminal buds also present distinct lumina to within 20 or 30 micra of their distal extremities. The lumen of each bud does not appear greater in diameter than that of other parts of the ducts but the walls of the bud are usually considerably thickened. In some places the lumina of the ducts at birth are partially filled with substance which resembles colostrum.

At the end of the first week, the lumen is considerably larger and extends farther into the intra-epidermal segment. Considerable quantities of the substance mentioned above are present in the lumina of all segments of the ducts.

Figure 16 shows that the lumen opens on the surface of the nipple at two weeks. At this stage it is larger and more oval throughout the primary duct than in the previously described stages.

In other stages up to the tenth week no marked differences were observed except that the lumina gradually become larger as the ducts increase in size (fig. 17). No true alveoli were observed, yet the end buds in the later stages are changed somewhat in appearance so as to approach, or at least suggest, beginning alveoli. In all the stages more or less of the substance mentioned in the earlier stages was observed. This agrees with findings of Berka ('11), who describes particles of fat in the human ducts even in the later virgin stages. He thinks the source of this fat is the colostrum secretion of the newborn and that it lies in the ducts for years.

4. Growth of the nipple

Owing perhaps to the fact that the rat is born in a somewhat immature state, the nipples show only slight development at the time of birth. In the newborn the nipple can be recognized by the naked eye only with difficulty, the nipple areas appearing slightly lighter than the surrounding tissue. A section through the nipple area at this stage reveals the fact that it is only very slightly elevated above the surface of the skin (fig. 14). The epithelium of this area is considerably thickened and the figure reveals an epithelial ingrowth on each side of the thickened portion. Reconstructions show that these epithelial projections are continuous around the nipple area and that through them a definite epithelial hood is formed, as shown in figures 3 to 6. Through this hood, which is filled with loose connective tissue, the primary duct passes on its way toward the surface.

Very little change takes place in the appearance of the nipple during the first week of postnatal life. From the surface the nipple areas appear slightly more elevated than at birth. Figure 15, drawn from a section cut very obliquely, shows that the thickened area of epithelium has become more extensive, also that the epithelial projections (ingrowth) are slightly longer.

During the second week the nipple develops very rapidly. It is no longer necessary to speak of it as the nipple area, since from its size and shape it now resembles a true nipple. Figure 16 shows the nipple at the age of two weeks to be a very prominent structure with the epithelial projections (ingrowth) correspondingly well developed. At this stage the primary duct actually pierces the surface. As previously stated, the nipples are very conspicuous externally toward the end of the second week of life, and from the tenth to the fourteenth days it was found easier to observe the glands externally than at any other time during the virgin state. During the third week, the nipples become hidden by the development of the hair coat.

Figure 16 also shows that at the age of two weeks a sulcus is beginning to appear between the nipple and the skin of the surrounding region. This sulcus is immediately superficial to the epithelial ingrowth mentioned above. Beyond the two-weeks stage (up to ten weeks) no changes have been observed to take place in the nipple, except that it slowly increases in size. This is shown in a nine-weeks stage (fig. 17) in which the structure is similar to that in figure 16, the chief difference being one of size.

SUMMARY

The results of the present investigation of the growth and gross relations of the ducts and the nipples of the albino rat from birth to the tenth week may be summarized as follows:

1. Observations made on 100 rats show the number of glands varies between 10 and 13, the normal number being 12 (6 pairs).

Only one supernumerary gland was observed. The second thoracic glands are those most often absent.

2. Only one primary duct is present in each gland. This duct after reaching the tela subcutanea turns at right angles and pursues a course parallel to the surface of the skin. The majority of the branches of ducts belonging to the first thoracic gland lie cephalad to the nipple, while those from the second and third thoracic glands lie latero-cephalad to the nipple. In case of the abdominal and first inguinal glands the greater number of the ducts lie latero-caudad to the nipples, while in the last inguinal the ducts lie caudad to the nipples. The dichotomous method of branching frequently occurs, especially in the proximal branches.

3. Reconstructions and cleared preparations show that anastomoses sometimes occur between the ducts of a single gland. It is uncertain whether anastomoses occur between the ducts of different glands.

4. End-buds are present on a large number of terminal ducts at all stages studied. No true alveoli were observed. Large numbers of lateral buds are present on the sides of all ducts distal to the primary duct during the earlier stages. Such lateral buds later develop into branches of the ducts.

5. Considerable individual variation in the development of the glands was noticed. Not only do the corresponding glands of opposite sides differ in their degree of development, but also the glands of one individual may be better developed than those of another even several days older.

6. The characteristic distribution and ramification of the ducts apparently depend upon the space available for their growth.

7. The growth and branching of the ducts goes on at an unusually rapid rate about the ninth week, probably corresponding to the age of puberty.

8. A distinct lumen is present at birth in all the ducts distal to the intra-epidermal portion of the primary duct. At the end of the second week the lumen extends to the surface of the nipple.

9. The periphery of the nipple area is marked by a hoodlike epithelial ingrowth. The nipples make the most rapid growth during the second week, toward the end of which time they are very conspicuous.

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PLATE 1

EXPLANATION OF FIGURES

7 Drawn from a cleared preparation (internal view) of an albino rat one week old (weight 8.5 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*); and left second inguinal gland (*C*). × 5. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct; *e.b.*, end-bud; *c*, collateral duct.

8 Same as figure 7, but from an albino rat two weeks old (weight 15.5 grams). × 5.

9 Same as figure 7, but from an albino rat three weeks old (weight 30 grams). × 5.

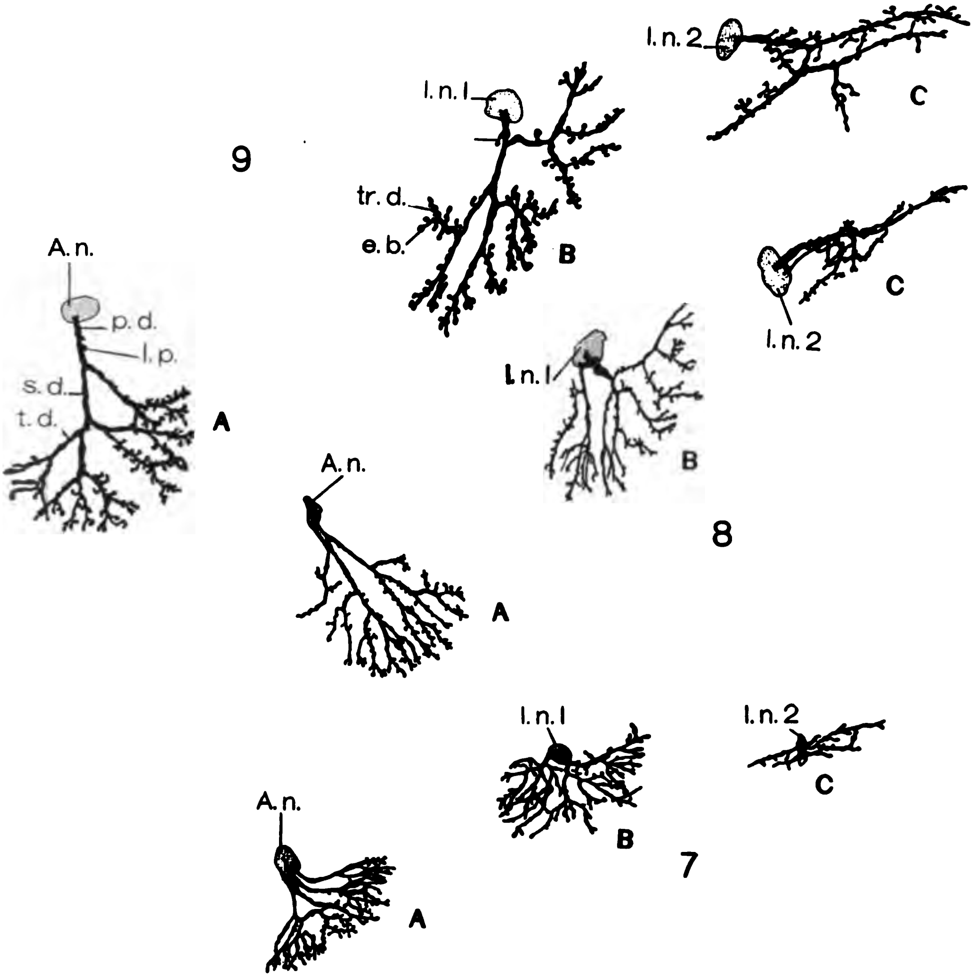

l. n. 2
C
l. n. 1
9
tr. d.
e. b.
B
C
l. n. 2
A. n.
p. d.
l. p.
s. d.
t. d.
A
l. n. 1
B
A. n.
A
8
l. n. 1
l. n. 2
C
B
A. n.
7
A

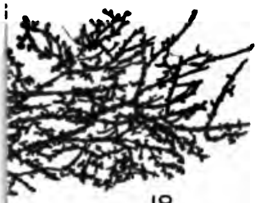
PLATE 2

EXPLANATION OF FIGURES

10 Drawn from a cleared preparation (internal view) of an albino rat four weeks old (weight 53 grams) to show distribution and relations of ducts of left abdominal gland (*A*); left first inguinal gland (*B*), and left second inguinal gland (*C*). × 5. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *tr.d.*, terminal duct; *e.b.*, end-bud; *L*, lymph-node.

11 Same as figure 10, but from an albino rat five weeks old (weight 54 grams). × 5.

PLATE 2



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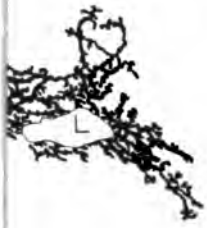

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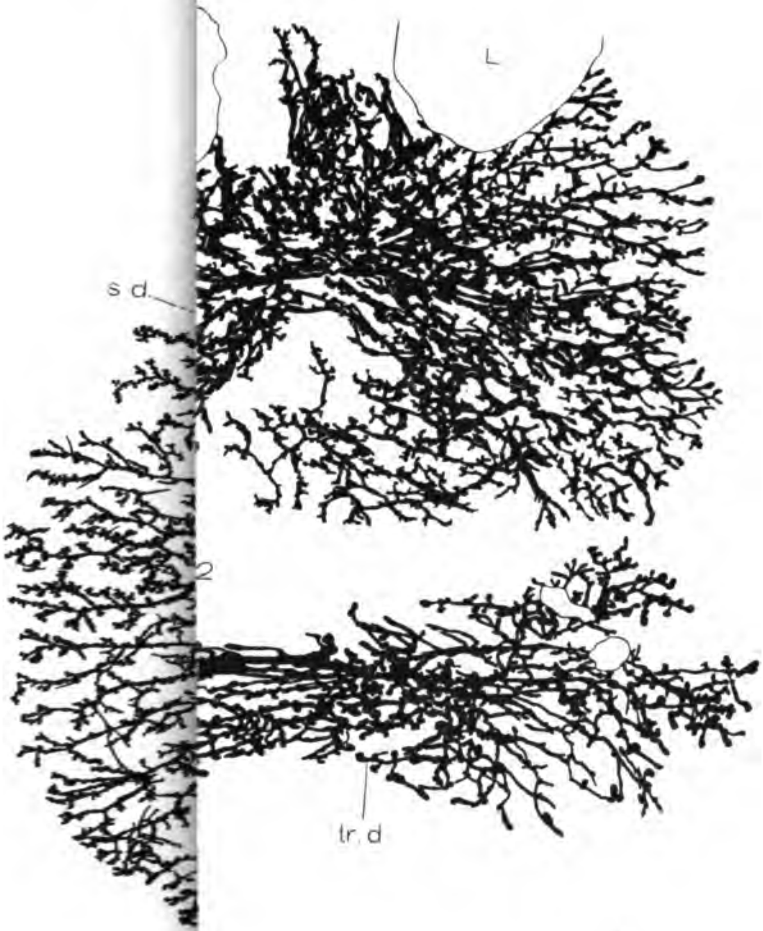
PLATE 3

EXPLANATION OF FIGURES

12 Drawn from a cleared preparation (internal view) of an albino rat seven weeks old (weight 75 grams) to show distribution and relations of ducts of left abdominal gland, first inguinal gland, and second inguinal gland. × 5. *A.n.*, abdominal nipple; *I.n.1*, *I.n.2*, 1st and 2d inguinal nipples; *L*, lymph-node; *p.d.*, primary duct; *s.d.*, secondary duct; *t.d.*, tertiary duct; *c*, collateral duct; *tr.d.*, terminal duct; *e.b.*, end-bud.

13 Same as figure 12, but from an albino rat nine weeks old. (Weight 114.5 grams.) × 5.



L
s d.
2
tr. d
m.
l.d.

PLATE 4

EXPLANATION OF FIGURES

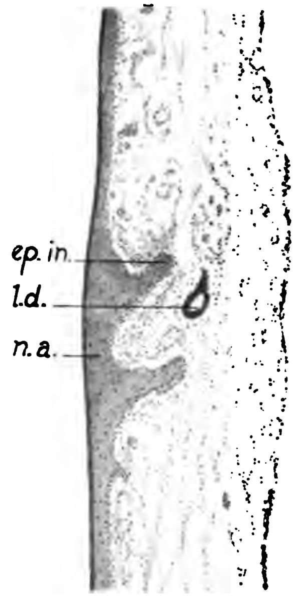
Lettering for figures 14 to 17 as follows: *ep.in.*, epithelial ingrowth of the nipple; *h.f.*, hair follicle; *l.d.*, lactiferous (primary) duct; *m.*, muscle; *n.a.*, nipple area; *s*, sulcus at base of nipple.

14 Drawn from a section through the nipple area of the second inguinal gland of a newborn albino rat. × 67.

15 Drawn from a section through the nipple area of the second inguinal gland of an albino rat one week old. × 67.

16 From a section through the nipple area of the second inguinal gland of an albino rat two weeks old. × 67.

17 From a section through the nipple of the second inguinal gland of an albino rat nine weeks old. × 67.



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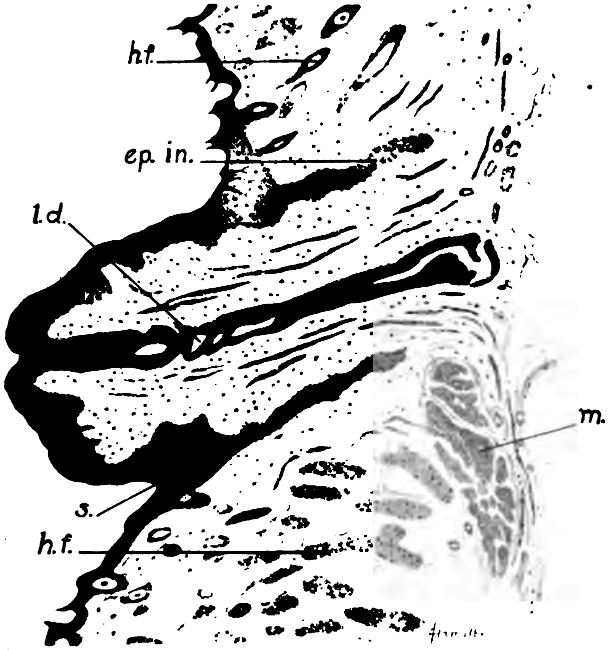

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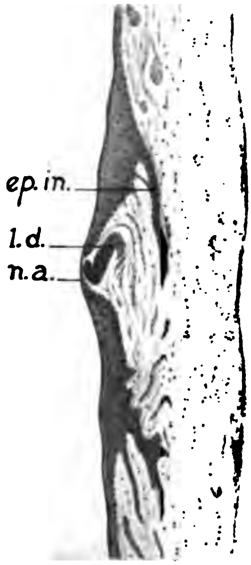

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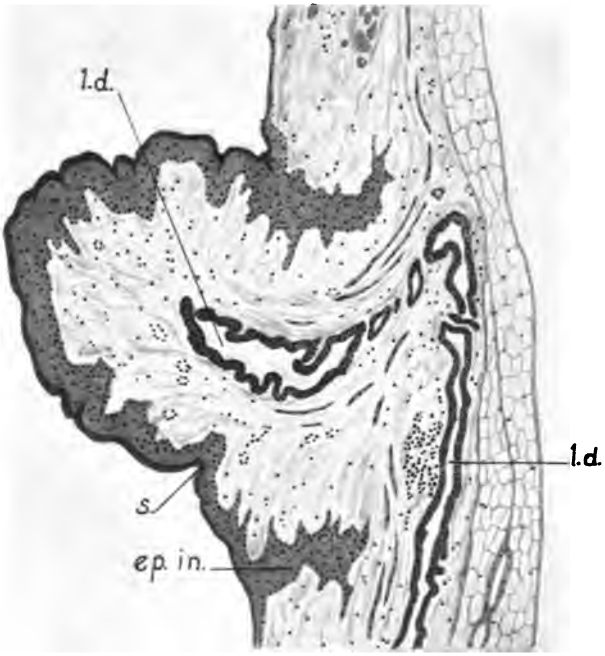

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THE MORPHOGENESIS OF THE FOLLICLES IN THE HUMAN THYROID GLAND

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SEVENTEEN FIGURES

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I. INTRODUCTION

In spite of numerous investigations, many questions concerning the development of the thyroid gland are still unsettled. This applies particularly to the morphogenesis of the thyroid follicles. The various and contradictory views in the literature on the development of the follicles are doubtless due in part to lack of adequate series of successive embryonic stages (especially of human embryos) available for study. The greatest difficulty, however, has arisen from the use of inadequate methods of investigation. The method hitherto almost exclusively used, that of direct observation of the microscopic sections, is insufficient. By the use of reconstruction methods, however, it has been found possible in the present study to reach a satisfactory solution of this difficult and important problem, at least regarding some of the more fundamental features.

This study was undertaken in the Anatomical Laboratory of the University of Minnesota at the suggestion of Prof. C. M. Jackson, under whose supervision the work was conducted. I wish to thank Dr. Jackson for his valuable aid and criticism.

II. LITERATURE

The literature concerning the thyroid follicle will be considered in chronological order. First, it is desirable to mention briefly the various views which have been held concerning the morphology of the adult (human) thyroid follicle. Then follows a brief statement of the conclusions concerning follicle development (including also prefollicular stages), which have been arrived at by the observers who have worked upon human material. A few observations, made on lower forms, which have seemed especially pertinent to the present problem, are also included. Unless otherwise indicated, however, all statements refer to human material.

The follicular structure of the adult human thyroid gland has long been known. According to Boéchat ('73), Lalouette (1750), who was the first to describe the minute structure of the thyroid gland, found vesicles which seemed to communicate with each other. Bardeleben ('41) is said by Zeiss ('77) to have been the first to describe the adult thyroid follicles as isolated structures. Five years earlier, however, Jones ('36) described the thyroid follicles in considerable detail as completely closed vesicles. Although there has been considerable disagreement concerning the structure of the adult thyroid follicle, the majority of the later observers have, like Jones, described the vesicles of the adult gland as closed, spheroidal bodies. Cruveilhier ('43), Virchow ('63), and more recently Boéchat ('73), Zeiss ('77), and Hitzig ('94), however, have followed Lalouette in describing the follicles as forming a system of branched and communicating cavities within the gland. Still others, like Streiff ('97), have maintained that both branching forms and isolated vesicles occur in the adult gland.

Jones ('36), who was perhaps the first to describe the microscopic structure of the human fetal thyroid, found that in a fetus of four and one-half months the cells of the gland had become partially arranged into solid, globular masses; but no vesicles were observed at this stage.

Remak ('55) described in chick embryos in the wall of the primitive saccular, epithelial thyroid anlage the formation of thickenings which become separated and later give rise to the thyroid follicles. He also thought that the original saccular anlage might persist for some time and form new secondary vesicles by a process of constriction. He described similarly the origin of secondary follicles, both by constrictions and by solid budding, in the thyroid of pig fetuses four inches and above in length.

Peremeschko ('67) described the division of primary into secondary follicles in mammalian fetuses. Colloid is described as arising partly by secretion and partly by colloid metamorphosis of epithelial cells.

W. Müller ('71) described a developmental stage in which the thyroid consists of a network of cylindrical tubes. Such tubes were found in a 24 mm. fetus and in decreasing numbers in later fetuses and even in a three year old child. These tubes arise from solid epithelial cords by the development of a central lumen. The segmentation of the tubes with the formation of the gland-vesicles is produced by ingrowth from the mesoblast.

Horcicka ('80) found the thyroid gland of a four months' fetus to be made up for the most part of solid cell masses with a beginning of lumen formation in the central cells of these masses. Typical gland structure is found after the fifth fetal month.

Wölfler ('80) described the formation of follicles from solid masses of epithelial cells. Toward the end of the fetal period and after birth the peripheral cells of the groups dispose themselves in a circle. The central cells become at first granular, then degenerate and disappear in the pale, granular mass which fills the lumen of the vesicle thus formed.

L. Stieda ('81) noted that the anastomosing epithelial cords ('Epithelstränge') of the embryonic, mammalian thyroid are at first always solid, but that in the ends of these cords lumina appear, and the resultant vesicles are gradually constricted off.

Baber ('81) described the fully formed follicle as spheroidal in form, but observed also branching follicles which are probably giving rise to secondary follicles by a process of division.

Wölfler ('83) described the process of later development in the human thyroid gland as centrifugal. He distinguished a cortical and a medullary portion, which are respectively youngest and oldest, least developed and most developed portions.

His ('85) described in the thyroid gland of an embryo (Zw) between 16.5 and 22 mm. in length cells grouped to form acini or tubes. The inner ends of the cells have a light, colloidal appearance.

Biondi ('89) found that the (postnatal) thyroid vesicle discharges its contents, collapses and finally rearranges itself in the form of a number of small acini which repeat the process. He held that the colloid arises by cell secretion, and not by cell degeneration.

Ribbert ('89) described a centrifugal growth of the thyroid in embryos and newborn. Follicles are formed by the outgrowth of solid buds or sprouts from the old follicles.

Lustig ('91), who studied the thyroid gland in the pig and other animals, affirmed that colloid and follicles appear synchronously as the result of the degeneration of the central cells of the preëxisting solid masses.

Podack ('92) found well formed follicles in a fetus of five months. In some parts of the gland the follicular structure is only suggested and many cell-masses and cell-cords are present.

Marshall ('93) found that the thyroid in chick and frog embryos presents a stage in which the gland is made up of communicating, epithelial tubes. In the rabbit he described the presence of out-growths, some solid and some hollow, from the primitive epithelial anlage. In the human embryo: "At an early stage the lobes are excavated by a number of detached cavities, which become the vesicles of the adult thyroid."

Zielinska ('94) found the structure of the thyroid in newborn children variable both in size and number of the follicles, and also in the amount of solid cell masses. The relations "errinern an acinöse Drüsen und erwecken den Gedanken, dass hier ein sich verästelnder Drüsenkanal vorliegt, als dessen Endbläschen die solide Zellhaufen gelten können."

Hürthle ('94) described in the thyroid of young dogs scattered masses of interfollicular epithelium, in which new (primary) follicles arise by the secretion of colloid into the angles between adjacent cells.

Anderson ('94) described secondary follicles (postnatal) arising from the collapsed epithelium of emptied follicles in various mammals. The new lumina are formed by cell-secretion of chromophile spherules.

According to L. R. Müller ('96), the origin of small secondary follicles from the larger follicles, as described by Ribbert ('89) is clearly evident, even in the human adult.

Tourneux and Verdun ('97) in a careful study of the branchial derivatives in the human embryo described the transformation of the (median) thyroid plate (and later of the lateral thyroid anlage) into a richly anastomosing network of solid epithelial cords by ingrowth of vascular connective tissue in a 14 mm. embryo. This network was likewise observed in embryos of 19 mm. to 37 mm. in length. At 37 mm., the cords become varicose, and follicles develop by the formation of a cavity within each of the enlargements. A similar process of morphogenesis is described in the rabbit embryo by Soulié and Verdun ('97).

Streiff ('97) made wax reconstructions of normal, adult human thyroid tissue, and found it to be made up of closed follicles, ovoidal or spindle shaped. Branched forms due to budding or to secondary fusion were also described; some of these more complex forms he thought may represent persistent branching, a continuation of the embryonic process. He concluded that the thyroid arises as a branched tubular gland, the follicles being formed by constriction of the tubes.

Schreiber ('98), in a fetus of three months, found the thyroid gland for the most part arranged into follicles which contained much colloid.

Kürsteiner ('99) in fetuses from 8 to 30 cm. in length found the thyroid lobules made up of round or elongated, solid or hollow follicles. The lumina are few in number up to about 20 cm., but in the older fetuses they are numerous and evenly distributed throughout the gland. Some branching vesicles were also noted at 17.5 cm.

Prenant ('01) (p. 13) stated that in the embryonic thyroid the solid epithelial cords are transformed into a network of tubes from which the follicles arise by a process of constriction.

Von Ebner ('02) found numerous well developed follicles in older fetuses and newborn. Between the follicles are found, even in the adult, frequent solid strings and nests of epithelial cells, which are in the majority during development.

Elkes ('03), who studied the thyroid in fetuses from four and one-half to six and one-half months in age, found that it presents both solid cords and well developed follicles in variable number. In the newborn the earlier follicles have largely disappeared, leaving only a few at the periphery of the gland.

Hertwig ('10) (pp. 444–446) described in the embryonic thyroid anastomosing epithelial cylinders. These become tubular; varicose dilations are by ingrowth of the adjacent connective tissue cut off to form the permanant follicles.

Isenschmid ('10) found that in the thyroid of children the gland grows not only by the increase in the size of the follicles, but by the formation of new follicles by two methods: budding and division. He found no evidence that follicles are formed from solid cell-masses (interfollicular epithelium) retained from the embryonal period.

According to Hesselberg ('10): "Die Ausbildung der Thyreoidea in der fötalen Periode erfolgt durch Zerfall der ursprünglich soliden Zellplatte in solide Zellstränge. Diese schnüren sich zu Bläschen ab, die zuerst am kaudalen Pol auftreten." The normal structure of the thyroid is established from the fourth fetal month on. Desquamation of epithelial cells was found in about half of the cases from the seventh to the ninth fetal month, and the follicles are almost entirely obliterated in the newborn. During the first week of postnatal life the follicles are reformed and increase in number by a process of budding.

Prenant and Bouin ('11) give an account of the development of the median thyroid anlage similar to that given by Prenant ('01).

Broman ('11) described in the differentiation of the thyroid anlage a tubular stage transformed by constrictions into beaded chains and finally into separate follicles.

According to Grosser ('12), the thyroid anlage begins to separate into solid cords in the human embryo of 8 mm. In the 50 mm. fetus the cords, especially in the periphery, appear beaded. The beaded cords become divided into separate cell-masses, the anlages of the follicles. The lumina may appear as independent cavities (no tubular stage), before the follicles are detached, or they may arise later even in early postnatal life.

Simpson ('12) referred to the tubular structure of the thyroid and describes the gland of a seven months old child as tubular in character.

Aschoff ('13) stated that in the developing thyroid gland connective tissue separates round epithelial balls from the anastomosing cords, and it is in these 'balls' that the follicular lumina develop.

Sobotta ('15) described the first lumina as appearing in the peripheral parts of the lateral thyroid lobes in a fetus 50 mm. in length. The final breaking up of the cell cords into single groups, which will later form follicles, progresses very gradually, so that the final structure of the gland is arrived at only after birth. Interfollicular epithelium persists, which may later give rise to follicles.

Kingsbury ('15) in describing the early development of the thyroid states that lumina (follicular cavities) appear within the cell cords in fetuses of 32 mm., but colloid is not demonstrable until 40 mm.

III. MATERIAL AND METHODS

This study is based upon the collection of human embryos in the Anatomical Laboratory of the University of Minnesota. Several of the series used are in excellent condition for histological study. The embryos of the collection have been variously fixed and stained. The additional glands specially prepared have, for the most part, been fixed in Formol-Zenker; embedded in paraffin, sectioned at 10 μ; mounted serially and stained with alum-haematoxylin and eosin, or iron-haematoxylin and eosin.

Besides the specimens mounted in the collection, other glands from newborn children and from children in the early years of life were obtained at autopsy. These were used merely for purposes of comparison, and have neither been listed below, nor discussed in this paper.

The following table shows the materials used in this work. The embryos and fetuses used are arranged in the order of their crown-rump lengths. 'Minn. E. C.' refers to the Minnesota Embryological Collection. An asterisk (*) following the number signifies that the thyroid gland alone was sectioned. Otherwise the entire embryo was available in serial sections.

The ordinary reconstruction methods, both plastic (Born's wax-plate method) and graphic, were utilized in the present study. In all cases where a determination of the follicular form or structure was attempted, special precautions were observed in making the reconstructions as accurate as possible.

The drawings for reconstruction were made with the camera lucida on transparent paper. After the drawings were completed, those of successive sections were superimposed upon a tracing-table, and each epithelial structure in the section given a letter or number. The drawings were controlled by careful microscopic observations, to determine the frequently complicated relations of neighboring follicles. By this method it was possible to determine with certainty what the limits of any particular mass or follicle might be.

SERIAL NO.	MINN. E. C. NO.	C. R. LENGTH IN MM.	FIXATION	SECTION THICKNESS IN MICRONS
1	H 6	6.0	Zenker	10
2	H 13	7.5	Alcohol	15
3	H 60	11.0	Alcohol	20
4	H 68	11.0	Bouin	15
5	H 134	12.0	Alcohol	20
6	H 1	15.0	Alcohol	12
7	H 23	15.0	Alcohol	10
8	H 18	15.5	Formalin	10
9	H 28	16.0	Alcohol	10
10	H 62	16.0	Formalin	20
11	H 58	17.0	Formalin	20
12	H 260	18.0	Formalin	15
13	H 24	19.0	Zenker	12
14	H 2	20.0	Picro-sulphuric	15
15	H 7	20.0		10
16	H 265	21.0	Formalin	20
17	H 3	22.0	Zenker	12
18	H 15	22.0	Alcohol	12
19	H 64	23.0	Alcohol-formalin	20
20	H 304	24.0	Zenker	20
21	H 56	24.0	Alcohol	20
22	H 5	25.0	Zenker	15
23	H 21	26.0	Alcohol	15
24	H 29	26.0	Alcohol	12
25	H 99	26.0	Alcohol-formalin	20
26	H 48	27.0	Bouin	12
27	H 10	29.0	Alcohol	15
28	H 375	29.0	Formalin	10
29	H 259	30.0	Formalin	20
30	H 108	30.0	Zenker	10
31	H 57	31.0	Formalin	20
32	H 16	33.0	Zenker	15
33	H 313	35.0	Formalin	20
34	H 122	39.0	Formalin	20
35	H 8	41.0	Formalin	12
36	H 12	41.0	Zenker	15
37	H 121	46.0	Formalin	60
38	H 115	50.0	Formalin	40
39*	H 290	56.0	Formalin	10
40*	H 85	60.0	Formalin	10
41	H 26	65.0	Formalin	30
42*	H 285	66.0	Formalin	10
43*	H 81	83.0	Alcohol	10
44*	H 286	86.0	Zenker	10
45*	H 75	90.0	Formalin	10
46*	H 267	115.0	Formalin	10
47*	H 34	120.0	Formalin	10
48*	H 49	126.0	Bouin	10
49*	H 187	158.0	Formalin	10
50*	H 381	163.0	Formol-Zenker	10

IV. MORPHOGENESIS OF THE THYROID FOLLICLE

a. Prefollicular period

A brief consideration of the thyroid gland in the prefollicular period is essential to an understanding of the follicular development. For it is during this prefollicular period that the anlages of the primitive follicles are derived from the original epithelial mass; and, to a certain extent at least, the size, form, and arrangement of the earlier follicles are thereby predetermined.

The earlier well-known stages in the development of the (median) thyroid anlage in the human embryo are not considered in the present paper. The original thyroid diverticulum becomes detached and transformed into a small epithelial plate, well shown in the 6 mm. embryo (No. 1 of the present series). This and several succeeding prefollicular stages of the thyroid were carefully reconstructed by Born's wax-plate method, but for the present purposes it is unnecessary to figure or describe these models.

As is well known, the (median) thyroid epithelial plate soon presents irregularities, as shown in the 7.5 mm. embryo (No. 2), and rapidly becomes transformed into what appears in cross-sections to be (as heretofore almost universally described) a network of anastomosing epithelial cords.

With the details of this process of transformation the present study is not concerned. One feature of the end result, however, which comes out clearly in the reconstructed models, is that the cord-like appearance seen in the sections is largely an illusion. Fundamentally the plate-like structure of the thyroid anlage persists for a considerable time, although somewhat modified by a complicated process of fenestration, splitting, and budding during the growth of the primitive epithelial plate. The resultant structure consists essentially in a mass of irregular, branching and fenestrated plates, for the most part longitudinally arranged (parallel to the long axis of the body), so that in cross-section they appear as 'cords' of epithelial cells (fig. 1). This type of structure, with varying degrees of complexity is found in the various prefollicular stages of embryos from about 10 mm.

to 22 mm. in length. (Numbers 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 of the present series.)

Such a description, however, does not apply to the so-called lateral thyroid anlage (ultimobranchial body). As described by Tourneaux and Verdun ('97) and others, this body long remains

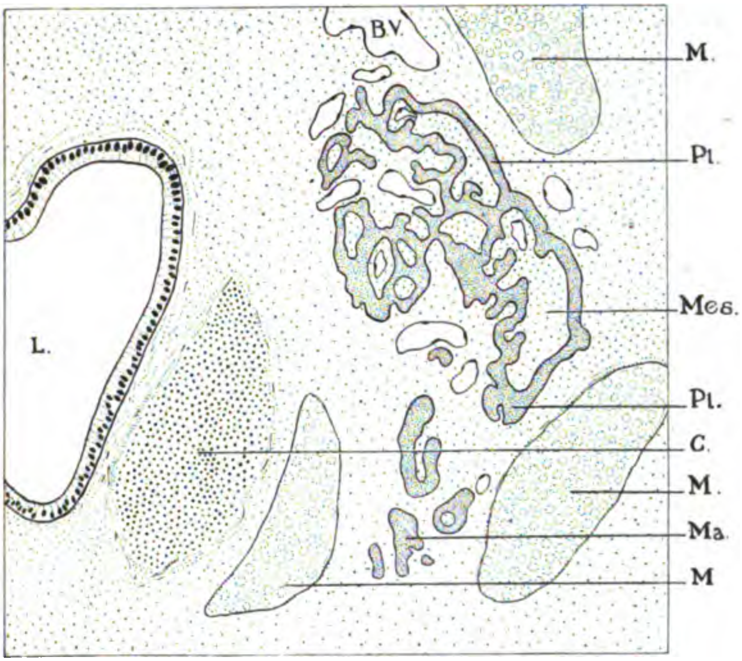


Fig. 1 Semi-diagrammatic drawing (camera lucida outlines) of a transverse section through the left lateral lobe of the thyroid gland and neighboring structures from a human fetus 22 mm. long (No. 18). This shows the complex interrelation of the smooth epithelial plates (*Pl.*) (which appear as 'cords' in the section) and the vascular mesenchyme (*Mes.*) found in the interspaces between them. The apparently detached epithelial masses (*Ma.*) shown are sections through projections from plates located in the upper part of the isthmus of the gland. *B.V.*, blood vessel; *M.*, muscle; *L.*, larynx; *C.*, cartilage. × 133.

as a compact, deeply staining mass of epithelium (the 'inner condensation' of Kingsbury '14) on the dorso-medial aspect of each lateral lobe. Whether in the human thyroid the lateral anlage later atrophies or finally becomes transformed into permanent thyroid tissue is still uncertain. This region is therefore purposely excluded from the following descriptions, which apply primarily to the process of morphogenesis in the structures derived from the median thyroid anlage.

The thyroid gland in the final stage of the prefollicular period is shown in a fetus of 22 mm. (No. 18). This stage will be described in some detail, in order to make clear the subsequent process of morphogenesis of the thyroid follicles. When studied in sections, the thyroid at this stage is seen to be made up apparently of epithelial 'cords' which are interrelated in a complex manner (fig. 1). A loose, anastomosing network is thus formed, the interspaces of which are filled with a vascular mesenchymal tissue (fig. 1). The 'cords' are, in general, only two cells in width, a feature characteristic of the thyroid plate and its derivatives at various stages.

Upon reconstruction (figs. 11 and 12) it is found that the epithelial network seen in sections, and described by so many observers as consisting of 'rods' or 'cords,' is merely a section of flat, slab-like plates, or bands, representing portions of fenestrated plates. It is true that some few of the epithelial masses are actually cord-like in form, but by far the greater number are better described as bands or plates. A definition of terms is necessary at this point. The term 'plate' will be used to signify a structure of relatively slight thickness, presenting expansive surfaces which are more or less smooth, and which may or may not be perforated (fenestrated). A band is a narrow plate. Therefore a fenestrated plate may be considered as made up of a number of anastomosing bands. There is great variability in the way in which these plates and bands are arranged. Some are mere slabs, which may or may not be perforated; others form irregular prisms or rounded cylinders, which open at both ends into the surrounding mesenchyme. The length and width of these plates is also quite variable. In three respects, however, they are in general agreement. They are two cells thick; present fairly smooth surfaces; and are longitudinally placed. Within these epithelial bands (fenestrated plates) the primitive follicles of the thyroid gland develop.

b. Follicular period

A thyroid follicle will be defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells. This definition includes all the features of the follicle which may be regarded as absolute and constant. The size and shape of the follicle may vary, and great differences are found in these respects in follicles of the same gland as well as in follicles of fetuses at different stages. Typically the thyroid follicle may be considered spherical or spheroidal in shape; but, as will appear later, this type is subject to considerable variation. The term primary follicle will be used to include those follicles developing independently of preexisting follicles. The follicles derived from preexisting follicles, by budding or otherwise, are termed secondary follicles.

In the present series, the first primary thyroid follicles appear in a fetus 24 mm. in crown-rump length (No. 20). In this fetus the thyroid gland has essentially the same structure as has that of the last (No. 18) described in the prefollicular period, i.e. it is made up chiefly of longitudinally placed epithelial plates or bands, only two cells in thickness. But in this stage, the plates, which have in previous stages been characterized by comparatively smooth surfaces, now present surfaces which are more or less roughened by the appearance of scattered hillocks or mounds (figs. 13 and 14). They are placed very irregularly with respect to one another, and may appear for the first time in any part of a plate, at its periphery or in a more central region.

When studied in cross sections (figs. 2 and 3) it is found that these hillocks are the immediate anlages of the early thyroid follicles. It is further seen that the hillocks are apparently produced by the concurrence of four different processes in the epithelium. The first process is that of cell rearrangement, the second that of cell proliferation, the third that of absolute cell growth, and the fourth that of lumen formation. These processes, although described separately, may occur simultaneously.

The first departure from the two-celled plate arrangement, in the process of follicle formation, is found in a rearrangement of the cells of the plate (fig. 3). The cell outlines can be made out only with difficulty in most cases. But in those places where they can be seen, they bound cells which are more or less columnar in form. The nuclei are ovoidal or elliptical in outline and are placed with their long axes perpendicular to the surface of the plate. Here and there along the course of the plate (fig. 3) it will be noticed that some of the nuclei have shifted their axes and have changed their relative positions. Certain of the nuclei have rotated through an arc of 90 degrees so that their

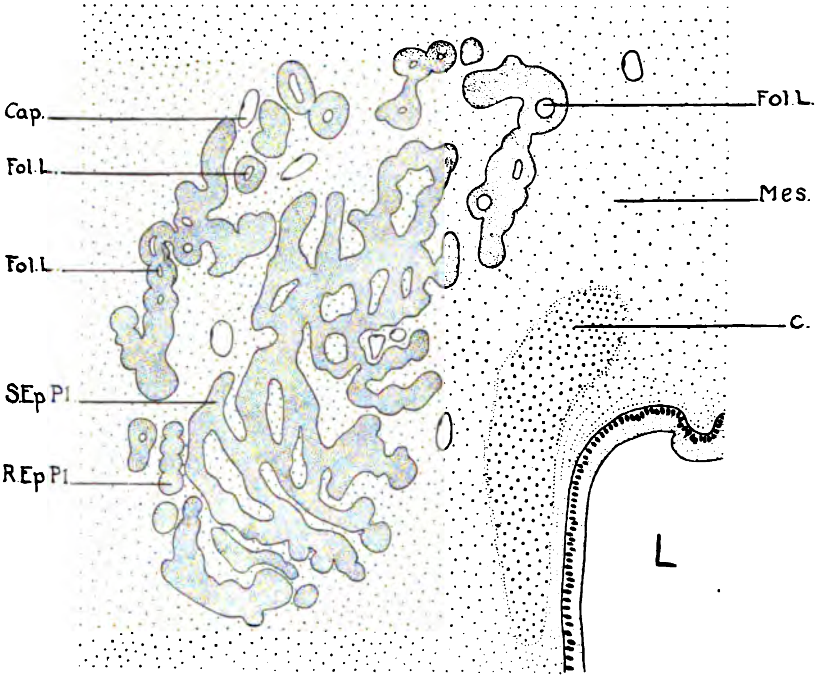
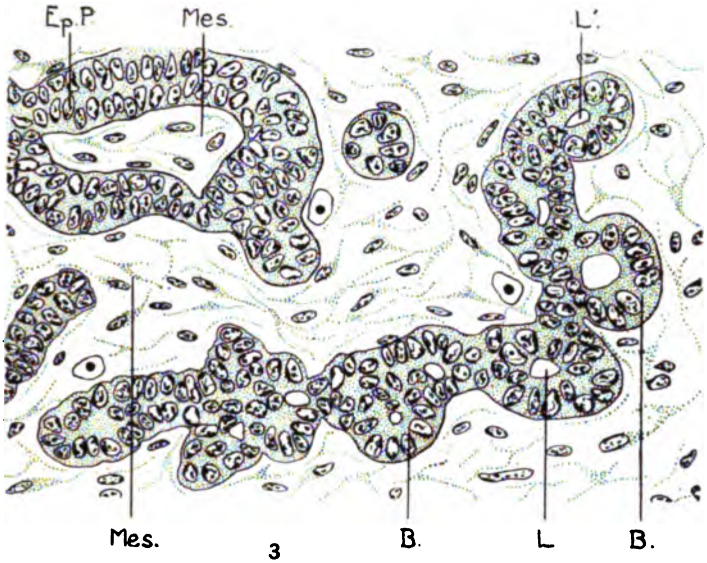


Fig. 2 Semi-diagrammatic drawing (camera lucida outlines) of a section through the right lateral lobe of the thyroid gland and neighboring structures of a human fetus 30 mm. long (No. 30). Shows epithelial plates developing rough surfaces; also early follicles in various stages of separation from the plates. Note the general increase in width of the plates as compared with those shown in figure 1, and the swellings and constrictions, giving 'beaded chain' appearance. *Cap.*, capillary; *Fol.L.*, follicle lumen; *S.Ep.Pl.*, smooth epithelial plate; *R.Ep.Pl.*, rough epithelial plate; *Mes.*, mesenchyme; *C.*, cartilage; *L.*, larynx. × 133.



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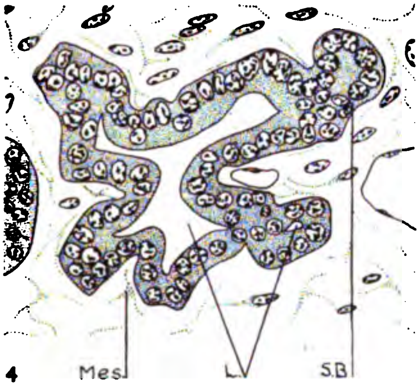

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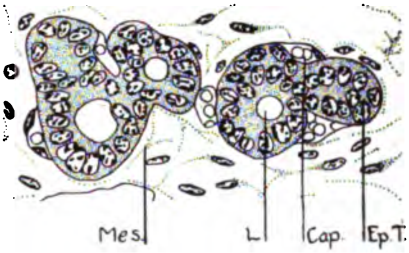

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Fig. 3 Small portion of a cross section of the thyroid gland in a human fetus 30 mm. long (No. 30), magnified to show the cell structure. The location of the section is indicated (*a–b*) in figure 14. Note the appearance of lumina (*L.*) in buds (*B.*) from the surface of the plate as well as in swellings along its course (*L'*). In the upper left hand corner a section through one of the hollow epithelial prisms is seen (*Ep.P.*). *Mes.*, mesenchyme. × 400.

Fig. 4 Drawing of a section through follicle '*d*' in figure 16. Note the irregular form of the follicle, both the lumen (*L.*) and the wall. *Mes.*, mesenchyme; *S.B.*, solid bud. × 400.

Fig. 5 Drawing of a section through an epithelial plate taken at the level marked *a–b* in figure 7. *Mes.*, mesenchyme; *L.*, lumen; *Cap.*, capillary; *Ep.T.*, epithelial tag. × 400.

long axes, in their final position, are at right angles to their original position in the plate. As a result of this shifting process, little circlets (really spheres) of nuclei are formed in the plate.

This shifting of nuclei is but the visible expression of the changed position of the cell. For while it is impossible to observe the cell boundaries in most cases, it is hardly probable that the nuclei shift their axes independent of the cytoplasm; moreover, the few faint cell-boundaries which may be made out show the same changes in position as do the nuclei. Further, it is usually found that at the point from which a nucleus has shifted toward the center of the plate a slight depression appears on the surface of the plate, indicating that the cytoplasm has shared equally with the nucleus in the movement. From these three facts it may be concluded that the first process manifested in follicle formation is the shifting of the axes of certain cells of the epithelial plate through an arc of 90 degrees. There is no evidence that the depressions on the surface of the epithelial plate are due to invasions or activity of the adjacent mesenchyme (fig. 3).

This process results in the transformation of the smooth surfaces of the bands (fenestrated plates) into surfaces which are somewhat roughened. The irregularities are apparently not, at first, due to swellings on the plates, but rather to the slight indentations produced by the shifting of certain cells toward the center of the plate as above described. When studied in cross sections (fig. 2) such a plate appears as a sort of beaded chain, with alternate swellings and constrictions. But, as noted above, the initial swellings due to this process are only apparent and are actually not greater in thickness than is the plate in other parts of its extent where indentations have not yet occurred.

The extraordinary cellular activity of the epithelium at this stage is clearly manifested by the large number of mitotic figures to be seen. There is no section of the gland in the 24 mm. fetus (No. 20) which does not present several cells in process of mitosis. But the localization of these mitoses is even more significant than is their number. It will be noticed that the nuclear figures are usually found only in those places in the epithelial plates where actual thickenings on the plates are being formed. Therefore the little mounds which appear on the plates, as the immediate anlages of the early follicles, may be formed not only by the rearrangement of the already existing cells of the epithelial plates, but also by the formation of new cells as well. Consequently, it can easily be seen how the apparent swellings on the plates, produced by the rearrangement of the existing cells, may be transformed into actual swellings, by the absolute increase in number of the cells found in a localized area. These swellings become roughly spheroidal in form.

The third process referred to above is the absolute increase in size of the cells. While the cells are shifting their axes and proliferating, they are also growing in size. This fact results in the appearance seen in figure 2, where the solid, two-celled plates are found in some cases to be no greater in cross section than the one-celled plate which surrounds the follicle lumen. It might be thought that the cells do not actually increase in size, but only increase in height by a closer crowding together. But a study of figure 3 will show that such is not the case. For the cells are not more closely packed together in the newly formed follicles than they are in the two-celled plate.

This progressive increase in the height of the cells corresponds to the progressive stages in the differentiation of the two-celled plate into newly formed follicles. So that the thyroid gland of a 30 mm. fetus (No. 30) presents in different regions epithelial cells varying greatly in height. The lowest cells are found at the beginning of the process, in the two-celled plate; the highest being found at the other extreme, in the completely formed follicle.

Three of the four processes above mentioned as apparently involved in the evolution of the follicle from the epithelial plate have now been reviewed in detail. The formation of the lumen remains to be considered. Just preceding the appearance of the lumen, the spherule (in which it is about to develop) appears in cross section as a circlet of very tall columnar cells, whose nuclei are peripherally placed. This arrangement results in the formation of a striking picture. The nuclei are regularly placed at the periphery of the circle and form a dark band, which surrounds an expansive, clear, central cytoplasmic portion. The magnitude of this cytoplasmic area and the sharp contrast between the two portions (in the stained preparations) are usually striking features (fig. 3). It is in the center of this cytoplasmic area that the lumen makes its appearance as a tiny spherical space outlined by a definite and regular margin. It is as though the cells had but drawn a little apart, so that their central ends, instead of remaining in contact one with another, might be separated by an interval. The relation of the early lumina to one another is well shown in figure 6, which is a graphic reconstruction of a plate. It is important to note that no tubular stage is found in the process of lumen formation. The lumina appear as absolutely independent spaces.

As the lumina first appear they apparently have no content; but undoubtedly they contain some substance which is not stained with the ordinary methods, and which increases in amount with the size of the follicular cavity. Certain of the larger lumina (not all of them), which are perhaps older, are found to contain a hazy, granular substance. Typical colloid does not appear until later, in the 60 mm. stage (No. 40).

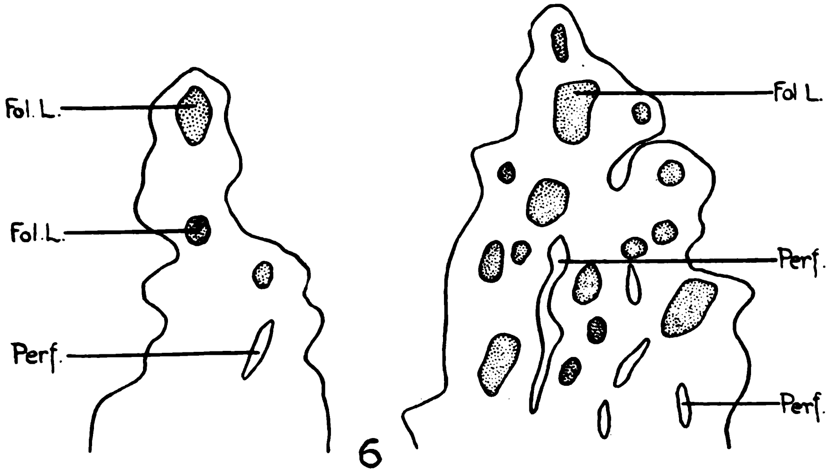
The various possibilities as to methods by which the follicular lumen may arise will be considered later in the discussion.

Fig. 6 A graphic reconstruction (surface view) of parts of two fenestrated epithelial plates from the thyroid gland of a human fetus 30 mm. long (No. 30) to show the relative position of the lumina (*Fol.L.*) as they appear in the plate. Lumina indicated by stippled areas. Note that in all cases these early lumina are quite distinct and never connected with one another. *Perf.*, perforation. × 267.

Fig. 7 A graphic reconstruction of a number of epithelial masses from the thyroid gland of a human fetus 60 mm. long (No. 40). Note the various degrees in the breaking up of the plates and the relative positions of the lumina. *a–b* indicates level of section in figure 5. × 267.

Fig. 8 A graphic reconstruction of a follicular complex shown in the lower part of figure 17. Lumina indicated by stippled areas. Note the solid buds. × 267.

Fig. 9 Model (reconstructed by Born's wax-plate method) of a large follicle from the peripheral part of the lateral lobe of the thyroid gland in a human fetus 60 mm. long (No. 40). The three buds shown have lumina communicating with the lumen of the main follicle. × 267.



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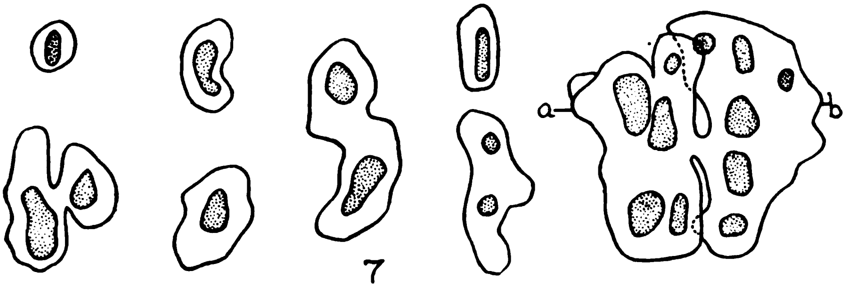

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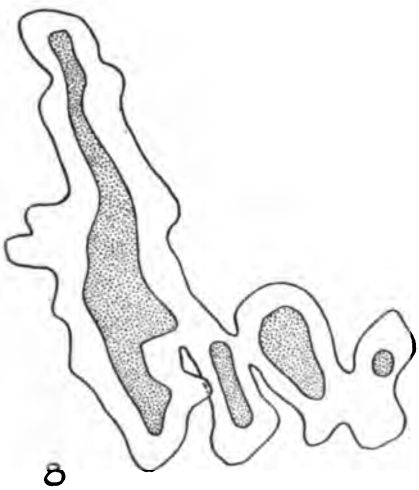

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From the time of their first appearance, considerable variability in the size of the lumina found in any particular gland is to be noted. The first isolated follicles are found in the more peripheral parts of the thyroid gland, and it is in these regions that they first attain large size and considerable complexity. For some time after the formation of follicles has begun, all stages previously described may be found in different parts of the same gland, a considerable portion of which retains the irregular plate-like type of structure characteristic of the prefollicular stages.

In the foregoing account, the cell masses in which the lumina develop have been described as spherules whose cross section is circular in outline. While this is true for typical follicles and in most cases, some variation within comparatively narrow limits is found. Ovoidal or somewhat irregular follicles occur, but these are not more numerous than would be expected in a rapidly growing tissue.

The foregoing descriptions of the early primary follicles have been taken in large part from observations made on two fetuses, one of 24 mm. (No. 20) and one of 30 mm. (No. 30). The excellent condition of these specimens has made possible studies of considerable detail. The members of the series (Nos. 21, 22, 23, 24, 25, 26, 27, 28, 29) intervening between these two, although not favorable for such intensive studies, show substantially similar structure. These stages may be summarized very briefly. The comparatively smooth epithelial plates of the prefollicular period have been transformed into plates with rough surfaces. The roughenings on the plates are the early indication of the follicles about to be formed. With the progressively increasing number of follicles the plates are transformed into irregular bands, which in turn give rise to groups of solid or hollow masses of cells. In the 30 mm. stage, however, the thyroid gland is still largely made up of anastomosing bands (fenestrated plates) (figs. 3, 6, 13, 14), although some entirely isolated follicles are found.

While the fetus is increasing in length from 30 mm. to 50 mm. the thyroid gland presents merely a continuation of the process above described; so that by the time the 50 mm. stage is reached the band or plate formations are relatively insignificant, while the isolated epithelial masses make up the greater part of the organ. All stages in the breaking up of the bands or plates may still be found, however. At this stage (50 mm.) the gland is therefore characterized by the presence of few epithelial plates or bands, very many isolated, solid cell-masses (anlages of follicles) and relatively few well developed follicles.

At 56 mm. (No. 39) the thyroid gland presents slightly more follicles than at 50 mm. Not only are the follicles increased in number, but they also show some changes in both size and form. In the previous stages the follicles have ranged from 16 to 55 μ in diameter (average diameter about 40 μ), and have been typically spherical in form. But in this stage many of the follicles at the periphery of the gland have enlarged considerably and have departed from the more usual spherical form of previous stages. This condition is more strikingly seen in the next fetus of the series (60 mm., No. 40) where both increase in size and change in form of the follicles are very evident (fig. 15). The number of follicles at this stage appears about equal to the number of solid epithelial masses. Figure 7 is a graphic reconstruction of a number of epithelial masses from which follicles are being formed and of certain isolated follicles. A study of sections at this stage will show follicles situated at the peripheral portions of the lobe whose diameters are three or even four or five times as large as those of greatest magnitude located more centrally. The average diameter of the centrally placed follicles is about 15 microns, while the average diameter of the follicles in the peripheral regions is approximately 55 microns. There is much less absolute variation in the size of the central follicles than in those of the periphery. Centrally placed follicles range from 13 to 17 microns, while peripherally placed follicles range from 20 to 103 microns in diameter. It will further be seen that these same large peripheral follicles are quite irregular in form. Instead of being circular in outline, they have a more or less regularly elliptical or ovoidal form, and may present a number of solid or hollow buds. Figure 9 represents a reconstruction of a typical one of these large budding follicles. Figure 7 is a graphic reconstruction of a number of epithelial masses found in the thyroid gland of the 60 mm. fetus (No. 40) to demonstrate stages in the breaking up of the plates and the formation of isolated follicles, which are present at this time.

The 65 mm. fetus (No. 41) is the first member of the series in which practically no epithelial plates or bands are found. From this stage on through the remainder of the series the thyroid gland is made up almost entirely of independent, solid or hollow, more or less irregular epithelial cell-masses. The stages (Nos. 42 and 43) included between this embryo and the one of 86 mm. (No. 44) may be passed over briefly with the statement that the processes of budding and formation of new follicles as previously described are advancing rapidly parallel with the increasing length of the fetus.

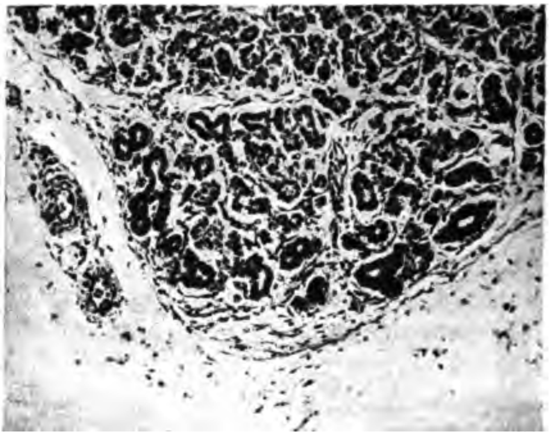


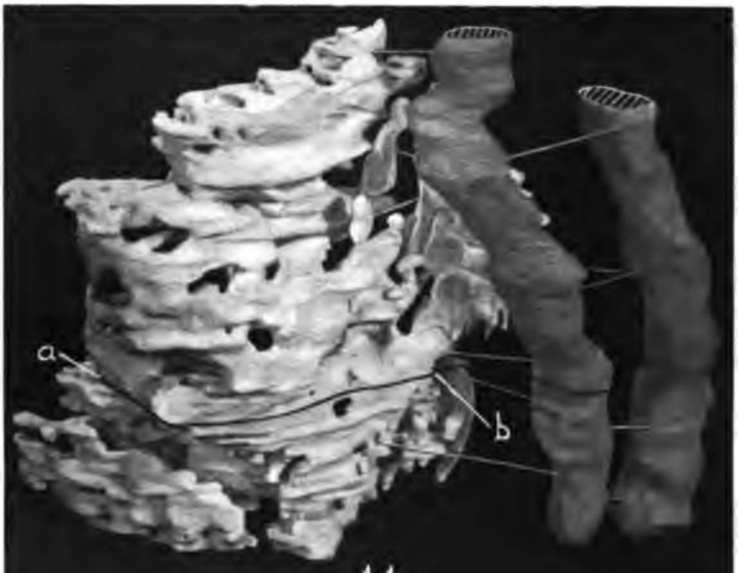
Fig. 10 Microphotograph of a portion of a cross section of the thyroid gland in a human fetus 86 mm. long (No. 44). Note the numerous branching follicles, also the elongated follicles in various stages of division by constriction. This section is in the region of the reconstruction shown in figure 15. × 100.

The 86 mm. fetus (No. 44) deserves special attention for it exhibits what appears to be the culmination of the budding processes referred to above. A study of figure 10 reveals the extreme complexity of the gland at this stage. The hollow and solid epithelial masses are about equal in number. The extreme variability in the form of the follicles is the most striking feature (figs. 4, 10 and 16), and the reconstructions reveal more clearly the complexity of these structures than is apparent in sections. Elongated follicles with numerous hollow or solid buds, as well as numerous varieties of other more or less complex arrangements are to be seen. The whole picture is one of active growth. There is great variability in the size of the follicles, the largest being for the most part located in the peripheral zone; but the extreme variation in the follicular form makes absolute measurements for comparison of follicles of little value.

This condition of the gland prevails in the remaining members of the series, up to and including the 158 mm. fetus (Nos. 45, 46, 47, 48, 49). There are but two differences to be noted. In the first place, the relative number of follicles is increasing from stage to stage, so that by the time the fetus is 158 mm. in length, the follicles in number are far in excess over the solid, interfollicular masses. Secondly, the number of irregular and complex forms, although still occurring, is becoming fewer (fig. 17), and the spheroidal follicles are relatively numerous.

The thyroid of the 163 mm. fetus (No. 50) has quite a different structure from that described for the members of the series just preceding (Nos. 44, 45, 46, 47, 48, 49). The irregular and branching follicular complexes are comparatively few in number, while small, spherical follicles make up almost the whole of the tissue. There are, however, a few solid, interfollicular epithelial masses still present at this stage. The picture has changed from one in which the epithelial structures, instead of being complex in their form, have become relatively simpler in character and for the most part are organized into small follicles.

My material from this stage on up to and including newborn children shows to a greater or less extent the process of epithelial desquamation in the thyroid follicles as described by Elkes ('03), Hesselberg ('10), Isenschmid ('10) and others. Whether this process is physiological or pathological is as yet undeter-



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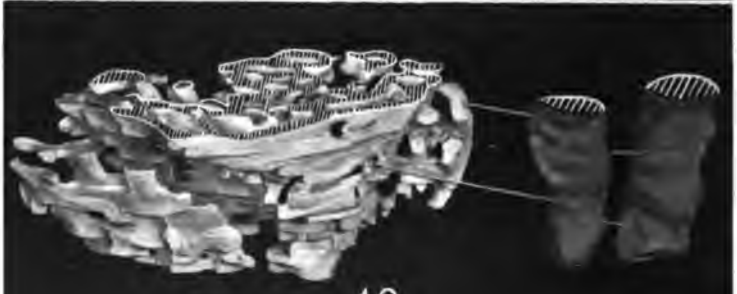

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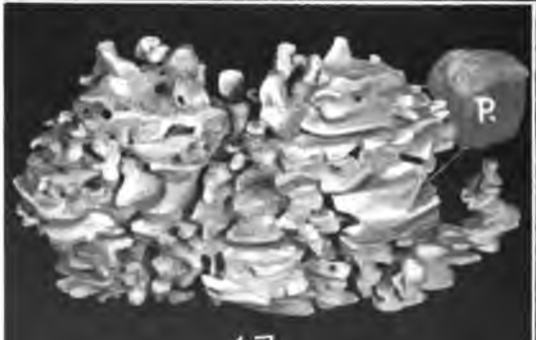

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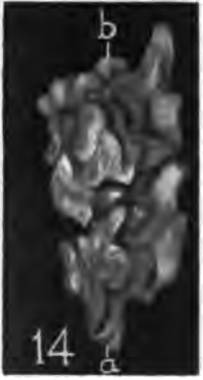

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mined. Because the material at hand is not sufficient to warrant the drawing of any conclusions in this matter, it is not listed with the materials nor discussed in this paper.

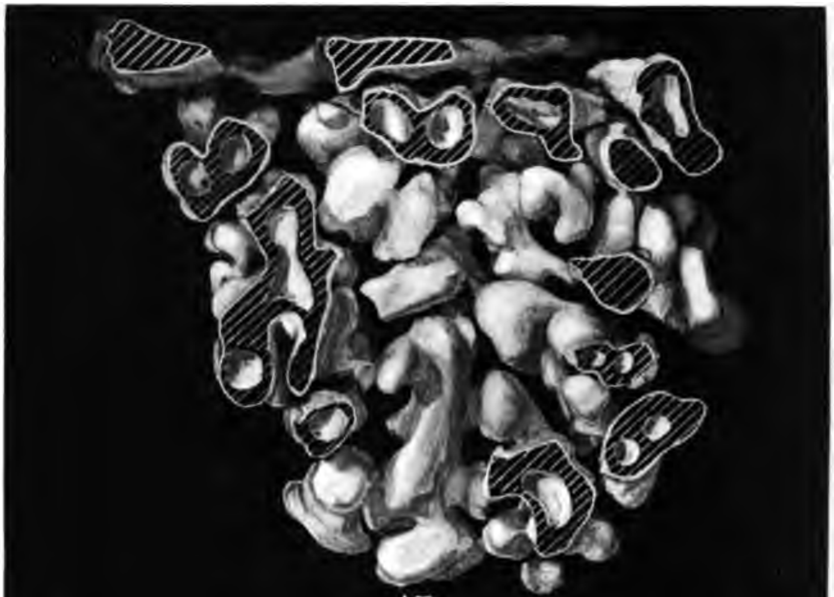
In four of the fetuses studied (Nos. 40, 42, 43, and 44) a number of cyst-like follicles located in all cases in the lower and posterior (dorsal) part of the lateral lobe, were observed. Some of these follicles measure as much as 200 microns in diameter. The size of these structures is not much greater than that of some of the larger normally appearing follicles. But in structure they are quite different, having walls made up of very much flattened epithelial cells, whose nuclei cause the cells to bulge and are separated from one another by much greater distances than is the case with the nuclei in the more usual follicles. The lumina of these cysts are quite regularly circular in outline and in many cases contain a granular substance. It is as though a follicle had been greatly distended, the cells of the wall being stretched and flattened.

Fig. 11 Model (reconstructed by Born's wax-plate method) of the left lateral lobe of the thyroid gland from a human fetus 22 mm. long (No. 18). Antero-lateral surface view to show the perforated (fenestrated) plates, which present a relatively smooth surface. Line *a–b* indicates the level of the section shown in figure 1, and the level at which the model was divided to show the relations pictured in figure 12. The carotid arteries are shown on the right hand side of the figure. × 150.

Fig. 12 The lower section of the model pictured in figure 11. The upper portion has been removed in order to demonstrate the relations to the gland mass of the appearances found in cross section. The separation of the two portions is made at the level of the section shown in figure 1 and indicated (*a–b*) in figure 11. × 150.

Fig. 13 Model (reconstructed by Born's wax-plate method) of the upper portion of the left lateral lobe of the thyroid gland from a human fetus 30 mm. long (No. 30). Plates are more broken up than in figure 11 and present surfaces which are relatively rough and irregular. The upper parathyroid (*P.*) is shown. Lateral view. × 100.

Fig. 14 Model (reconstructed by Born's wax-plate method) of a part of a plate from the thyroid gland of a human fetus 30 mm. long (No. 30) at a higher magnification, to demonstrate more exactly than shown in figure 13 the appearance and relations of the mounds forming on the surface of the plate. The plane *a–b* indicates the position of the section shown in figure 3. Lateral view. × 265.



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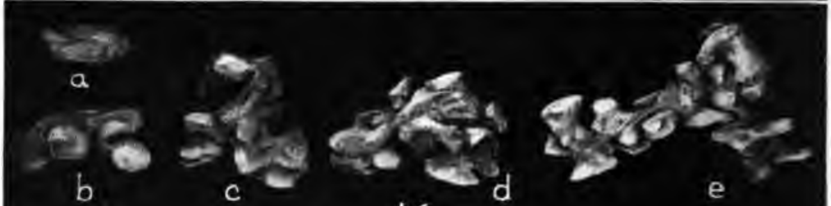

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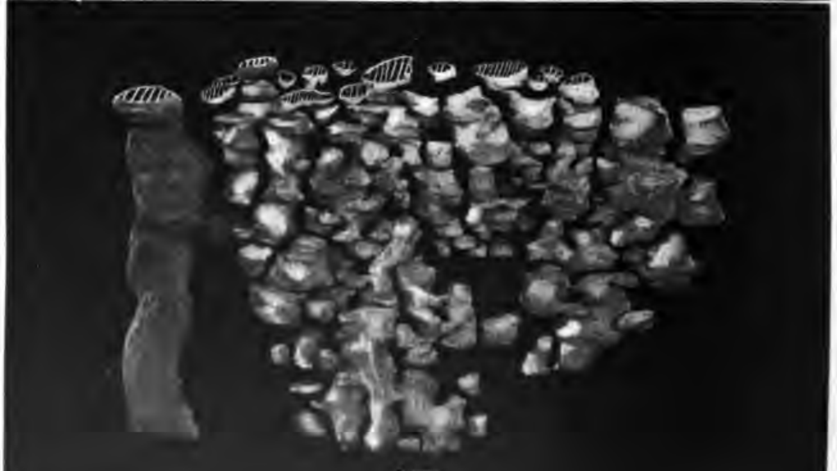

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V. DISCUSSION AND CONCLUSIONS

Remak's theory of the derivation of the thyroid follicles directly from the primitive saccular thyroid anlage has not been confirmed. In the prefollicular stages, the thyroid is by recent investigators quite generally described as assuming the form of irregular, anastomosing 'cords' or masses of epithelium. This undoubtedly appears to be the case when sections of the gland are observed (figs. 1 and 2). But the reconstruction methods used in the present investigation reveal a surprisingly different condition. It is found that, as a matter of fact, in the great majority of cases the cords are illusions and in reality are merely sections of fenestrated epithelial plates longitudinally arranged.

As to the further steps in the process of morphogenesis of the follicles from these anastomosing 'cords', widely divergent views have been held, as noted in the section on 'Literature.' While differing considerably in detail, these views may be classified according to their principal features. The morphogenesis of the primary follicles and of the secondary follicles will be considered in their order.

According to the apparently most widely accepted view, based perhaps largely upon preconceived ideas or theories concerning the evolution and morphogenesis of the thyroid gland, there are two distinct stages in the transformation of the epithelial 'cords' into follicles. First the anastomosing 'cords' acquire lumina, so that the gland becomes a more or less definite network of hollow epithelial tubes. The tubes then become constricted (by

Fig. 15 Wax reconstruction of a portion of a peripheral lobule of the thyroid gland of a human fetus 60 mm. long (No. 40). This model is from the region shown in figure 10. The structure placed across the top of the figure is a blood vessel. The oblique lines designate cut surfaces. Viewed from above. × 270.

Fig. 16 A series of follicles (*a*, *b*, *c*, *d*, *e*) reconstructed by Born's wax-plate method to show the varying degrees of complexity. All are taken from the thyroid gland of a human fetus 86 mm. long (No. 44). × 135.

Fig. 17 Wax reconstruction (Born's method) of a peripheral lobule of the thyroid gland in a human fetus 158 mm. long (No. 49). The variable form and mutual relations of the follicles are evident. The structure at the left of the figure is a blood vessel. The oblique lines designate cut surfaces. × 130.

ingrowth of vascular connective tissue) into spheroidal segments, each of which becomes a small sac or follicle whose cavity represents a portion of the originally continuous lumen of the tube. This view has been advocated by W. Müller ('71), Marshall ('93 (in chick and frog), Streiff ('97), Prenant ('01), Hertwig ('10), Prenant, Bouin and Maillard ('11), Broman ('11) and others. Some like Streiff ('97) and Simpson ('12) have described this branching tubular condition as persisting .in part throughout fetal life and even in postnatal life.

Other investigators, however, have described the lumina of the primary thyroid follicles as appearing directly and idependently, with no preceding tubular stage. The anastomosing solid cell-cords are usually described as becoming varicose, with successive enlargements and constrictions, so as to present an irregular beaded chain appearance. Sooner or later each of these spheroidal swollen masses acquires a lumen and becomes separated so as to form an independent follicle. This method of follicle formation (with no tubular stage) has been described by Tourneux and Verdun ('97), Soulié and Verdun ('97), Grosser ('12), Aschoff ('13), Sobotta ('15) and Kingsbury ('15).

It is impossible to decide from direct observations of sections which of the preceding theories is correct. By reconstruction methods, however, both graphic reconstruction and wax-plate models, evidence has been secured in the present investigation which definitely disproves the tubular theory and establishes in the human thyroid the independent origin of the lumina of the thyroid follicles. The follicles, however, appear not in epithelial 'cords' as described by earlier observers, but in the fenestrated epithelial plates above mentioned. The view that the thyroid is a modified branching tubular gland (Zielinska, Streiff, Simpson, and others) therefore obtains no support from its morphogenesis, aside from the initial stage of the primitive diverticulum.

In the glands studied in the present series the first follicles appear in a fetus of 24 mm. in length (No. 20). This is earlier than the time of appearance described by most observers. His ('85), however, described follicles in a fetus (Zw) whose absolute length is not recorded, but is placed in series between fetuses of 16.5 and 22.0 mm. in length. Kingsbury ('15) described follicles in a fetus of 32 mm.; Tourneux and Verdun ('97) in one of 32.4 mm.; and Grosser ('12) and Sobotta ('15) in fetuses of 50 mm. These are the only cases found in the literature where the presence of early follicles has been noted in fetuses of definite length. Several observers refer to the age of fetuses in which the thyroid follicles appear, but in terms too indefinite to be of value for comparison.

Although by definition the prefollicular period ends abruptly with the appearance of the first follicles, it is not true that the structure of the gland undergoes any corresponding sudden change with the advent of the follicular period. The epithelial bands (fenestrated plates) are only gradually replaced by the primary follicles and structures characteristic of the prefollicular period may be present through a considerable part of the follicular period, at least until the fetus has attained a length of 65 mm.

Concerning the first three processes (rearrangement of the cells, cell proliferation, and increase in the size of the cells) involved in the development of the follicles from the epithelial plate, no further discussion is necessary. The fourth process, however, that of lumen formation, calls for further consideration. The follicular lumen might arise in various ways, which have been suggested by earlier investigators. Most of the workers, however, do not mention the process by which the lumen of the follicle is formed.

Wölfler ('80) and Lustig ('91) have described the formation of lumina in the solid cell masses by a degeneration of the more centrally placed cells. The present investigation does not support this view, however. In the first place none of the so-called central cells have been found; and secondly no evidences of degeneration have been observed in the primary follicles.

It might be supposed, as Hürthle ('94) and Anderson ('94) have suggested, that the lumen is formed by the accumulation of colloid between the angles of the cells which compose the solid mass. Such a process would leave a colloid-containing space surrounded by epithelial cells. But in no case in the present observations was 'colloid' found within the very early follicles; although the accumulation of some other (precolloidal?) secretory product between the angles of the cells, might result in lumen formation. The fact is that the smaller lumina, which are probably the most recent in formation, have been universally found to be devoid of any demonstrable content, and that some of the larger and supposedly older lumina do contain a stainable substance. As to the nature of the precolloidal substance or substances, nothing definite is known.

One might suggest (as thought by His?) that the lumen could be formed by a degeneration or liquefaction of the central ends of the cells which later form its outline. In this case it would be expected that the early follicles would present a lumen outlined by an irregular or ragged margin. Without exception, however, the lumina of the early follicles are clearly outlined and marked off by a very sharp margin.

Having studied the way in which the follicle forms within the plate, it is of further interest to determine the method by which the follicle frees itself from the plate and comes to take up an isolated existence. Earlier observers, in describing the formation of follicles from anastomosing rods or tubes, have laid emphasis upon the activity of the adjacent mesenchymal tissue as the factor operating in the separation of the follicles. In the present study no definite morphological evidence appears in favor of this view. For as shown in figure 3 there is no special differentiation of the mesenchyme or increased vascularity in the regions in which follicles are being separated off from the plate. The evidence seems rather to indicate that the follicles themselves are the active agents in their separation. Thus as certain of the cells leave their original positions to assume a position more nearly in the center of the plate, the indentations previously described appear on the plate. These may be considered as weak points. And as the cells increase in number between these indentations it is not difficult to see how the increased pressure due to the increased mass might force certain follicles out from the row in which they formed and thus isolate them from the parent plate. The vascular mesenchyme doubtless takes some slight part in the process, however.

As previously pointed out, the primary follicles may not in every case be at once separated completely from the plate. Instead of being in all cases sharply outlined and spheroidal in form, small portions of the plate (epithelial tags) may be left hanging to the follicle wall. The significance of such epithelial tags is readily understood when it is considered how easily they might be mistaken for epithelial buds arising from the primary follicle in which secondary follicles were about to form. These structures will be mentioned later in the discussion of the secondary follicles. It has been seen that there is no sharp line of demarcation between the prefollicular and follicular periods. Similarly, in the origin of follicles, the period of primary follicle formation is not sharply marked off from that of secondary follicle formation.

The number of thyroid follicles is apparently not absolutely established or finally limited at any stage of development (as is the case, for instance, with the glomeruli of the kidneys). In the earlier stages, the number of follicles increases by the formation of additional primary follicles. Soon, however, these primary follicles begin to give rise to secondary follicles (at 56 mm.). In later stages the formation of primary follicles apparently ceases, although their occurrence even in the adult has been claimed, e.g., by Hürthle ('94) and Sobotta ('15), the newformed follicles being all secondary in character. Various methods of secondary follicle formation have been described.

1. Origin from buds or sprouts. Ribbert ('89), L. R. Müller ('96), Streiff ('97), Isenschmid ('10) and others have described this process. The bud is usually described as a local thickening of the follicle wall, which continues to increase in size by the proliferation of cells, until a solid bud, projecting into the stroma, is formed. Directly, through the concentric rearrangement of the cells, the form of the lumen can be made out, even while the young follicle is still in contact with the mother-follicle.

2. Origin from collapsed follicles. Biondi ('89) Anderson ('94), and others have described the process as follows. After filling the vesicle discharges its contents, collapses and finally rearranges itself in the form of a number of small acini which repeat the process.

3. *Origin by fusion of follicles.* Streiff ('97), v. Ebner ('02), Isenschmid ('10) and others have observed follicles which are apparently formed by the secondary fusion of two or more preexisting follicles.

4. *Origin by division of follicles.* Isenschmid ('10) has described the formation of daughter-follicles by the growth of an epithelial spur across the lumen of the mother follicle; and Peremeschko ('67) has noted the formation of secondary follicles by the constriction of the parent follicle.

In discussing my observations concerning the formation of secondary follicles in comparison with those of earlier observers, it may be said at the outset that nothing to support either the second or the third methods just outlined has been noted. These, however, have been described chiefly by previous observers upon postnatal material. The other two methods (budding and division of follicles), however, are in general agreement with the findings of the present investigation.

There appear to be three general methods by which secondary follicles arise in the fetal thyroid, the third of which might be regarded as a modification of the second. But each of these types is subject to a wide degree of variation, so that many intermediate and modified forms are found.

1. Solid epithelial buds may develop on the follicle wall (figs. 4, 8 and 16*d*). These may become separated from the parent follicle while in the solid state, or they may develop lumina while connected with the wall of the mother-follicle, and subsequently be constricted off. This method is essentially that advanced by Ribbert ('89), L. R. Müller ('96), Streiff ('97), and Isenschmid ('10). It is difficult, especially in the earlier stages, to distinguish these solid buds from the 'epithelial tags' representing persistent portions of the original epithelial plates remaining attached to the earlier primary follicles.

2. Hollow buds whose cavities are continuous with that of the mother lumen are also found (figs. 4, 9, 10). It might be sug-

gested that these were originally solid buds whose lumen appeared independently and later established a secondary connection with the lumen of the parent follicle. While it is difficult to disprove such an occurrence, it would tend to reduce rather than to increase the number of follicles and seems improbable. The parent follicle apparently sends off extensions or branches, which represent both the wall and the lumen and gradually become constricted off to form new follicles. All possible stages in such a process, from the slightest outpouching of the wall to the finally separated follicle are easily observable.

3. The third method of secondary follicle formation is the simple division of the parent follicle somewhat as described by Peremeschko. The process appears similar to that of hollow bud formation. The follicle first takes on the form of an elongated ellipse which becomes constricted about its center. No cases of division by the ingrowth of epithelial spurs as described by Isenschmid (for postnatal stages) have been observed in my fetal material, though such may possibly occur.

Having considered the primary and secondary follicles separately it remains to consider them in their relations to each other and to the gland as a whole.

The formation of the secondary thyroid follicles begins when the fetus has reached a length of about 56 mm. From the 70 mm. stage on the formation of secondary follicles progresses with such rapidity that the total number of follicles is very greatly increased within a short period of time, and the relative number of primary follicles becomes progressively smaller.

It might be thought that such elongated, irregular, branched and budding forms as appear in large numbers in certain of the older members of the series (especially in the 86 mm. fetus, No. 43) are derived from irregular, branching bands (fenestrated plates) retained from the prefollicular period. But the evidence disproves that view. After the appearance of the first follicles, the bands and plates are being progressively broken up until the gland parenchyma in a fetus of 65 mm. is practically devoid of such structures, and is almost entirely made up of isolated solid or hollow masses of cells. So closely does this resolution of the plates parallel the increasing length of the embryo up to 65 mm. that it is very improbable that individual variations could explain the presence of these structures in such great numbers at the relatively late stage of 86 mm. Further, as shown in figure 16, forms of all degrees of complexity may be found from simple spheroidal follicles to those of extreme complexity. The conclusion is therefore reached that these branching structures, which are better described as follicular complexes than as follicles, are developmentally only follicles which have grown excessively and attained a high degree of complexity.

Such a rapid increase in the number of thyroid follicles as occurs in fetuses between 65 and 158 mm. in length might be expected to produce a marked increase in the size of the gland. But according to Jackson ('09) the growth curve for the prenatal thyroid gland shows no remarkable increase in the size (weight) of the gland during this period. These two observations, which at first may appear contradictory, are readily explained when the size of the follicles is taken into account. The secondary follicles formed by budding and division of the primary follicles are very small and arise from follicles which are in most cases relatively of much greater size. So that while the number of follicles is greatly increased during this period, the gland mass is not correspondingly larger. As previously described, the formation of secondary follicles becomes less rapid before the fetus has reached 163 mm. in length. From this point on, the number of thyroid follicles apparently increases but slowly, the subsequent growth of the gland being due rather to the increase in the size of the individual follicles than to a further increase in their number.

The significance of the large cyst-like follicles described in four of the fetuses is uncertain. Kürsteiner ('99) has described the presence of similar follicles in four fetuses. The remarkable regularity with which they were found, in his cases as well as in those of the present series, located in the lower and posterior (dorsal) part of the lateral lobe of the gland, is a striking fact. Possibly they may be related to the cysts of the thyroid gland, frequently met in pathological conditions of postnatal life.

VI. SUMMARY

By methods of reconstruction (both graphic and wax-plate), the complicated process of morphogenesis of the follicles of the prenatal human thyroid gland has been worked out and several mooted points definitely established.

1. The so-called 'cords' forming the anastomosing network in sections of the thyroid (median anlage) in the later prefollicular stages represent chiefly sections of epithelial bands, two cells in thickness, and forming irregular, fenestrated plates.
2. The frequently described stage in which the 'cords' are transformed into an anastomosing set of epithelial tubes from which the follicles are derived does not exist. The process of follicle formation gives no evidence or indication that the thyroid has been derived from a branching tubular gland.
3. The primary thyroid follicles arise directly as isolated and independent structures from the epithelial plates of the prefollicular period, by the rearrangement of cells, cell proliferation, increase in the size of the cells, and lumen formation.
4. The primary follicles appear in fetuses about 24 mm. in length. The epithelial bands (fenestrated plates) have practically disappeared in a fetus of 65 mm., but a few solid interfollicular epithelial masses are still present in fetuses 163 mm. in crown-rump length.
5. Secondary thyroid follicles are formed from preexisting follicles apparently by three methods: by solid buds; by hollow buds; and by constriction of the parent follicle.
6. The first secondary follicles appear in fetuses about 56 mm. in length, but are formed most rapidly in stages when the fetus is between 80 mm. and 158 mm. long. After 163 mm., the growth of the gland probably takes place largely by the increase in size of the individual follicles, rather than by increase in their number.
7. Large cystic follicles were observed in the lower and posterior (dorsal) parts of four glands from the older fetuses. Their significance is uncertain, as is likewise the apparent involution of the follicles with desquamation of epithelium observed in the later fetal and newborn stages.

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ON THE DEVELOPMENT OF THE BILIARY SYSTEM IN ANIMALS LACKING A GALL-BLADDER IN POST-NATAL LIFE

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TEN FIGURES

It is well known that the gall-bladder is always absent in some species of vertebrates. There seems to be no general rule governing the distribution of this peculiarity. Thus in the cyclostomes a gall-bladder is found in the myxinoids but is absent in the fully developed Petromyzon. It is present as a rule in fishes although absent in a few species of sharks. A gall-bladder is said to be present always in amphibians and reptiles; it is wanting in a number of birds including the pigeons. Among mammals no gall-bladder is found in a number of rodents and in many ruminants, pachyderms and some other ungulates. It is absent in the Cetacea. In other forms the gall-bladder may or may not be present. This is the case in several species of birds and in a few mammals. With the latter may be included man where congenital absence of the gall-bladder is an unusual anomaly. In man the gall-bladder alone may be lacking, or more rarely both the gall-bladder and the larger biliary ducts.[2]

Only three of the forms consistently lacking a gall-bladder are commonly available for embryological study. These are the lamprey (Petromyzon), the pigeon and the rat. The development of the biliary system in these species will be taken up in the order given above.

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[1] This work was done with the aid of a grant from the Research Fund of the University of Minnesota.

[2] The comparative list given above is by no means a complete one. A much more detailed statement is to be found in the Milne Edwards, Lecons sur la physiologie et l'anatomie comparée, T. 6, p. 454, et seq. Later papers upon the partial or complete absence of the biliary system in man are: Hochstetter, Arch. f. Anat., 1886; Latham, Journ. of Anat., 1898; Bubenhofer, Anat. Hefte, 1905; and Beneke, Marburger Universitätsprogramm, 1905.

PETROMYZON

I have made no study of Petromyzon material but include here a brief summary of the findings reported in the literature.

The early history of the liver in Petromyzon has been studied by Götte ('90), v. Kupffer ('93), Brachet ('97) and others. A complete biliary system is developed including a large gall-bladder and a common bile-duct of considerable diameter. These structures are lined with a high columnar epithelium which later becomes somewhat flattened and they are surrounded by definite mesenchymal sheaths. The biliary apparatus takes part in the general rotation of the gut which occurs in this form and it persists during the Ammocoetes or larval period.

The later history of the common bile-duct and gall-bladder has been followed out in some detail by Nestler ('90). He found that in an Ammocoetes 13 cm. in length the bile-duct still opened into the anterior end of the mid-gut and was connected distally with the gall-bladder. Near its posterior or intestinal end there was a short segment in which the lumen was almost completely obliterated by the increased height of the lining epithelium. In a specimen a centimeter longer the common bile-duct was still connected with the gall-bladder but its connection with the intestine was lost and its caudal end obliterated. In this region was found a collection of 'Zellballen' or follicles which were connected with the part of the duct still intact. In still later stages the duct proper was found to have entirely disappeared but the follicles persisted. Nestler apparently regarded these follicles as cystic remains of the common bile-ducts or of outpouchings from it. The gall-bladder first became much reduced in size and the epithelium lining it increased in height. The surrounding sheath of mesenchymal tissue also was thickened. Later the structure was completely obliterated.

These observations were confirmed in the main by Bujor ('91) who, however, accepted the earlier view of Langerhans ('73) that the follicles observed near the cranial end of the common bile-duct were pancreatic. This view has been confirmed by the recent careful researches of Picqué ('13).[3]

PIGEON

In the pigeon, as in birds generally, there are two ducts leading from the liver to the intestine. Of these the anterior or cranial one is commonly termed the ductus hepato-entericus. The posterior one is divided into two parts by the point of attachment of the gall-bladder. The portion between the hepatic trabeculae and minor ducts and the gall-bladder is called the ductus hepato-cysticus, and the part between the gall-bladder and intestine the ductus cysticus or ductus cystico-entericus. Since the pigeon, as a rule at least, loses its gall-bladder in the course of development, the terminology above is not always an appropriate one. For simplicity the two main ducts will be designated in the following description as the anterior and posterior hepatic ducts.

Brouha ('98) has published the only information which we have concerning the later development of the liver in the pigeon. He modelled and described two stages, embryos of 59 and 100 hours incubation respectively, in connection with his extensive study of the development of the liver in the chick. The liver forms from the floor of the foregut and the anterior intestinal portal, but the anlage, which is hollow in the chick, is solid at first in the pigeon. In Brouha's younger specimen the anlage was already partially divided into anterior and posterior diverticula as in other birds. In the older specimen this division was quite distinct and the anlage of the gall-bladder was represented by a distinct expansion of the left side of the extreme posterior end of the hepatic gutter at the point of origin of the posterior hepatic diverticulum or duct (Brouha, '98, pl. VIII, fig. II, V. B.). Brouha did not follow its later history.

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[3] Although Nestler does not describe a pancreas in Petromyzon, he figures and labels as pancreas a group of tubules in the wall of the mid-gut on the side opposite to the follicles associated with the ductus choledochus.

The youngest pigeon embryo I have examined is 7 mm. in length, of 72 hours incubation (Minn. E. C. 588). In development it stands between the two stages described by Brouha. The anterior and posterior divisions of the hepatic diverticulum are differentiated into short wide ducts which connect with a considerable network of hepatic trabeculae surrounding, in part, the large right omphalo-mesenteric vein. All three pancreatic anlagen are present. The dorsal one forms a pouch which is almost as large in cross section as is the gut at this level. The ventral diverticula are smaller pouches which arise from the gut just above the posterior end of the hepatic gutter. A right lateral view of a reconstruction of this region is shown in figure 1. In the reconstruction the hepatic trabeculae are cut away near their origin from the hepatic diverticula. The posterior hepatic diverticulum is very short and ends by dividing into two parts. The right division becomes continuous with the hepatic trabeculae and represents the ductus hepato-cysticus of other

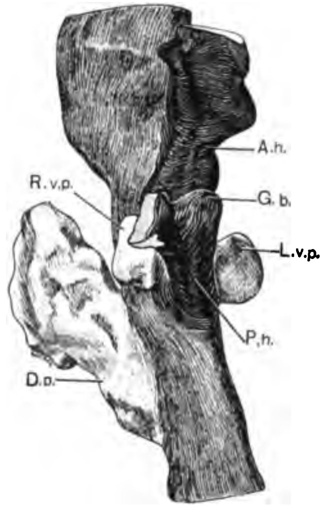

Fig. 1 A reconstruction of a portion of the mid-gut, liver and pancreas of a pigeon embryo 7 mm. long (Minn. E. C. 588). Right lateral view, × 50. *A.h.*, anterior hepatic duct; *D.p.*, dorsal pancreas; *G.b.*, gall-bladder; *L.v.p.*, left ventral pancreas; *P.h.*, posterior hepatic duct; *R.v.p.*, right ventral pancreas.

birds. The left division is the anlage of the gall-bladder and at this time is simply a short rounded swelling. This swelling is due almost entirely to the thickening of the epithelium, there being no true lumen in the cystic anlage (fig. 3).

The posterior hepatic diverticulum lengthens rapidly, extending forward and to the right. The cystic anlage is carried along with it and is soon located some distance from the gut in the margin of the hepatic parenchyma. The right branch of the bifurcation which connects with the hepatic trabeculae straightens out so that its axis falls in line with that of the main duct and the two form a single tube. The thickening representing the gall-bladder is strongly evaginated forming a small pouch which

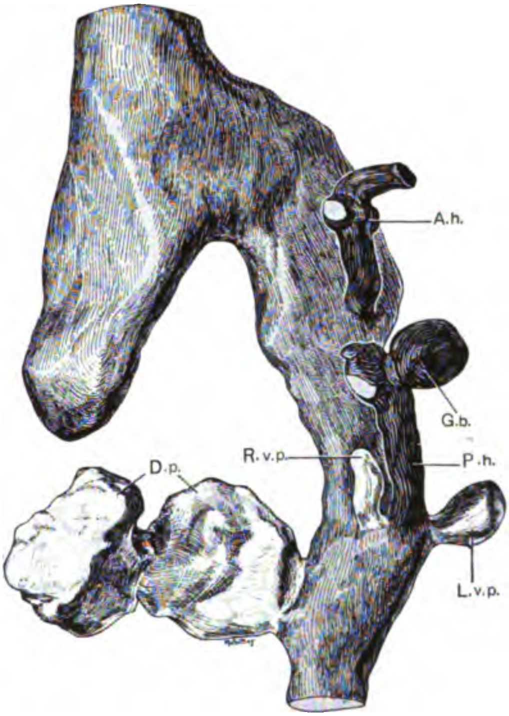

Fig. 2 A reconstruction of a portion of the mid-gut, liver and pancreas of a pigeon embryo 8 mm. long (Minn. E. C. 590). Right lateral view × 50. Abbreviations as for figure 1.

joins with the posterior hepatic duct by a short broad neck. Its walls become thinner and its lumen is thereby widened, but as a whole it increases very little in size. An embryo (Minn. E. C. 590), 8 mm. in length and distinctly older than the one first described, shows these changes in progress. Figure 2 is a right lateral view of a model of this specimen, and figure 4 a section through the long axis of the gall-bladder.

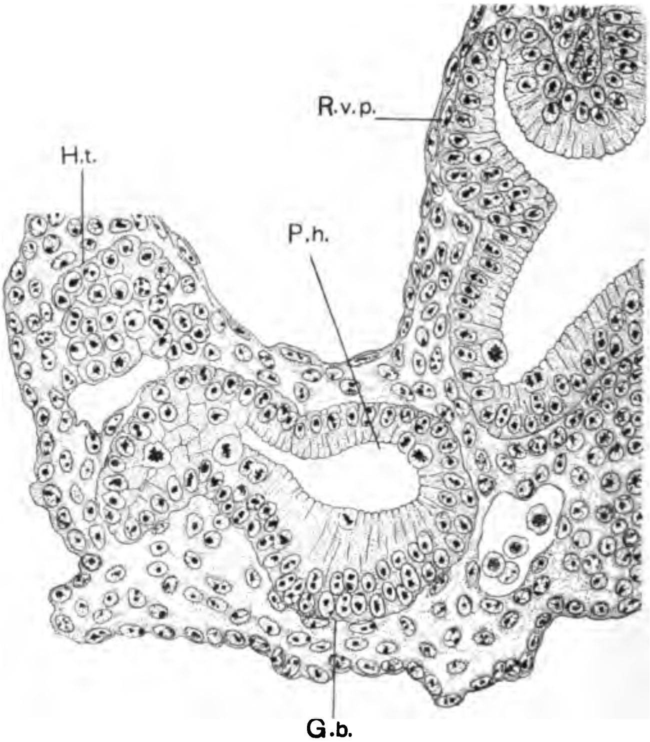

Fig. 3 Transverse section of the liver and the duodenal region of a pigeon embryo 7 mm. long (Minn. E. C. 588). × 400. *G.b.*, gall-bladder; *H.t.*, hepatic trabeculae; *P.h.*, posterior hepatic duct; *R.v.p.*, right ventral pancreas.

In later stages the entire mass of the liver is much enlarged. Both the anterior and posterior hepatic ducts increase rapidly in length and in caliber, but the anterior one grows more rapidly than the posterior. Although the liver and its ducts continue to increase in size, the gall-bladder remains about constant until the embryo reaches a length of 12 to 15 mm. Thereafter the gall-bladder becomes somewhat smaller both absolutely and relatively than in earlier stages. Both the lumen and the walls of the sac tend to diminish, and folds or sacculations may appear in its fundic end. The surrounding mesenchyma begins to take the form of a definite sheath with concentrically placed nuclei. The neck or true cystic duct becomes elongated and slender. Figure 5, an oblique section of the gall-bladder of an embryo 15.5 mm. long (Minn. E. C. 586), shows these changes in progress.

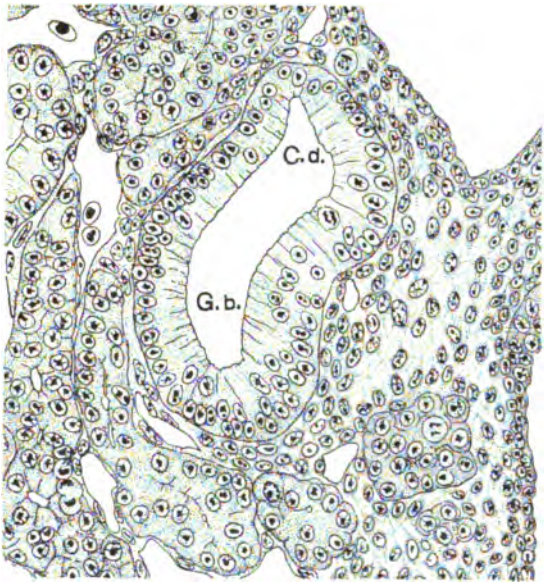

Fig. 4 Section through the long axis of the gall-bladder of a pigeon embryo 8 mm. long (Minn. E. C. 590). × 400. *C.d.*, cystic duct; *G.b.*, gall-bladder.

With the growth of the liver the gall-bladder changes its position, being carried away from the margin of the liver parenchyma where it was formerly located. In later stages it comes to lie more and more in the posterior part of the dorsal ligament.

The cystic duct is reduced in caliber and the lumen becomes very small or is occluded. From the specimens which I have examined it appears that the bladder generally becomes separated from the duct and remains for a period as an isolated, thick-walled cyst lying in the dorsal hepatic mesentery close to the posterior hepatic duct. This condition is shown in figure 6 of a section of the gall-bladder in an embryo 26.8 mm. long (Minn. E. C. 596).

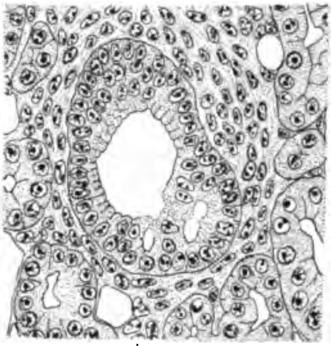

Fig. 5 Section through the long axis of the gall-bladder of a pigeon embryo 15.5 mm. long (Minn. E. C. 586). × 400.

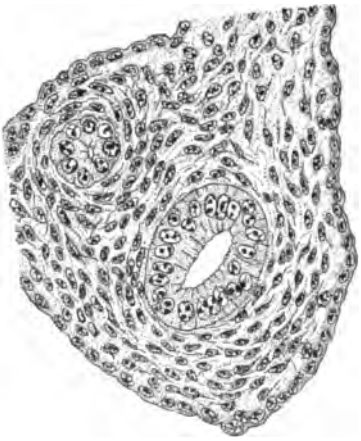

Fig. 6 Section through the gall-bladder and posterior hepatic duct of a pigeon embryo 26.8 mm. long (Minn. E. C. 596). × 400. The gall-bladder is the larger of the two epithelial tubes.

Later the gall-bladder degenerates in most cases. In an embryo 45 mm.[4] in length there was no definite cyst but in the region formerly occupied by the gall-bladder there was found a small cluster of deeply staining and degenerating cells which probably represented its remains. There was no trace of this structure in an embryo 55 mm. in length. A specimen 62 mm. in length, however, showed a small persistent gall-bladder. It was of about the same diameter as the duct to which it was attached and a common musculo-fibrous tunic surrounded the

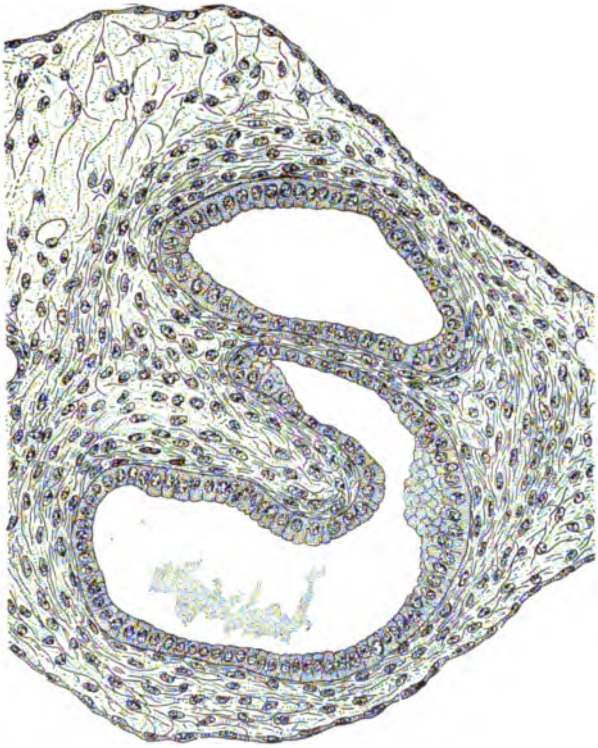

Fig. 7 Section through the posterior hepatic duct and gall-bladder of a pigeon embryo 62 mm. long. × 400.

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[4] These measurements are crown-rump length taken with the neck straightened out until in line with the body.

two structures. A slender and abruptly curved cystic duct connected the gall-bladder with the posterior hepatic duct.

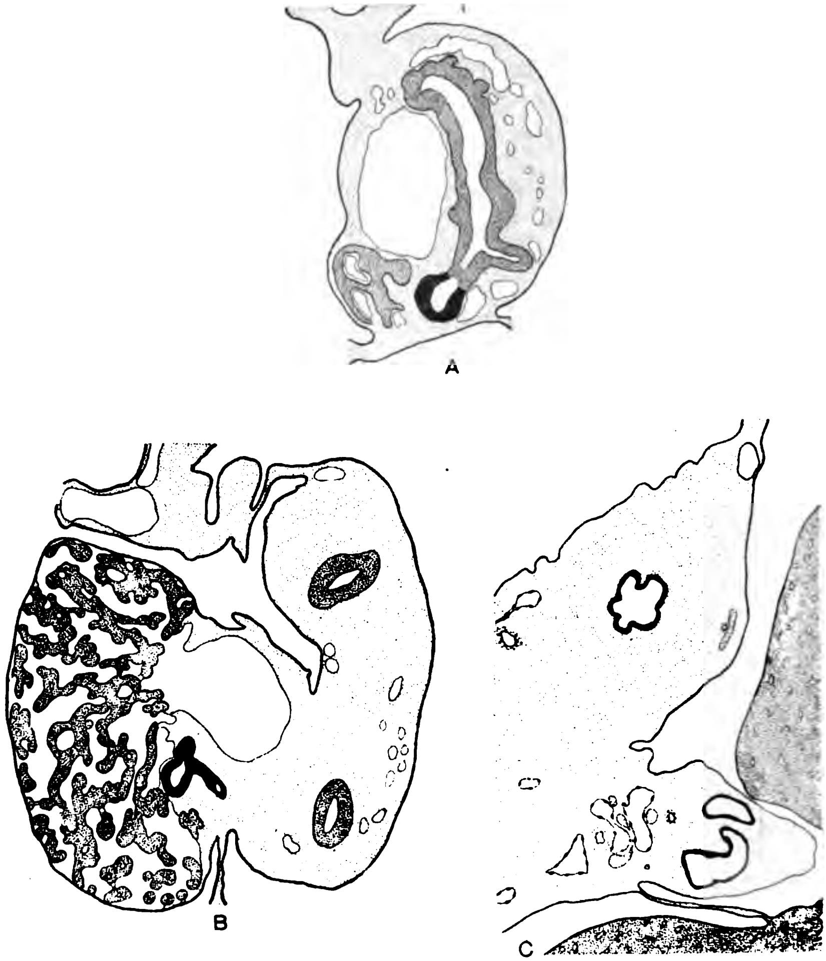

Fig. 8 Three sections showing the size of the gall-bladder in the pigeon embryo. *A*, embryo 6.8 mm. long (Minn. E. C. 591); *B*, embryo 8 mm. long (Minn. E. C. 554); *C*, embryo 62 mm. long. All × 50.

The lining epithelium of the bladder was of a low columnar type similar to that of the duct proper, and the lumen contained a small amount of granular precipitate (fig. 7). It is evident from this specimen that the cystic anlage may persist in some cases until about the time of hatching and perhaps later. The structure is so small that it could hardly be detected by dissection.

Attention has already been called to the great increase in the diameter of both hepatic ducts and the lack of corresponding growth of the gall-bladder. This is shown graphically in figures 8A, B and C which show longitudinal sections of the gall-bladder in embryos 6.7, 8 and 62 mm. in length respectively. These sections are all drawn at the same magnification.

RAT

Helly ('01) while studying the development of the pancreas in the rat examined the hepatic anlagen in an extensive series of embryos ranging from 2.2 mm. to 10 mm. in length. He states that in no specimen could he find an indubitable trace of a gall-bladder. He noted that in an embryo 2.8 mm. long the primitive ductus choledochus was of considerable caliber and that the ventral portion of its wall appeared somewhat thickened. But, as this thickening passed over directly into the sprouts of the hepatic trabeculae, he did not regard it as a clear anlage of a gall-bladder. Debeyre ('04), also working on the pancreas of the rat, studied a very complete series of embryos from 11 to 21 days old. He states definitely that he found no gall-bladder in an embryo 12 days old, and makes no mention of the structure in his description of later stages.

The youngest rat embryos which I have examined were 1.75 mm. in length and 11 days old (Wistar Inst. Coll. Nos. 15346 and 15347).[5] In these specimens the liver is already differentiated as a thickening of the ventral part of the foregut and anterior intestinal portal. The foregut is expanded a little ventrally forming a shallow hepatic pouch. From this pouch extend the earliest hepatic trabeculae which appear as solid sprouts of epithelial cells. Sagittal sections show that the liver is composed of two parts, an anterior, the true hepatic, from which the trabeculae are arising, and a posterior which gives rise to no trabeculae and which becomes continuous with the wall of the anterior intestinal portal. There is no evident cellular differentiation between these two parts aside from that associated with the formation of the hepatic trabeculae and the presence of a larger number of mitotic figures in the pars hepatica. A median section of the hepatic anlage at this stage is shown in figure 9.

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[5] My thanks are due to Dr. M. J. Greenman, Director of The Wistar Institute, and to Dr. C. A. Heuser, for their kindness in loaning me these preparations for a considerable period.

A specimen one day older and 3.36 mm. long (Wistar Inst. Coll. No. 15363) shows the liver anlage in the form of a distinct

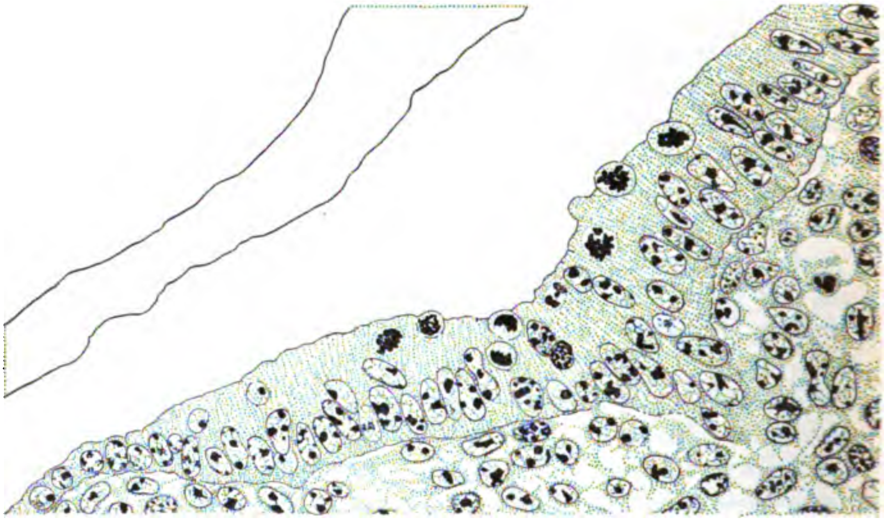

Fig. 9 Sagittal section of the hepatic pouch of an albino rat embryo 1.75 mm. long, 11 days, (Wistar Inst. Coll. No. 15346). × 400.

pouch sharply marked off from the gut anteriorly and posteriorly with the forward end somewhat recessed. A large number of looping and anastomosing trabeculae arise from the anterior part of the pouch and extend some distance into the surrounding mesenchyma (fig. 10). The posterior part of the pouch gives rise to no trabeculae. In the ventral wall of this portion just in front of the posterior end of the anlagen is a slight swelling which is marked on the internal surface by a shallow pit (*X*, fig. 10). It extends but a little way on either side of the mid-line.

The arrangement of the cells in this swelling suggests that seen in taste-buds but the cell boundaries are not distinct. The nuclei are elongated and peripherally placed while those of the remainder of the hepatic pouch are rounded and more generally scattered. The cytoplasm is a little lighter than that of the remainder of the pouch. This structure may be the trace of a gall-bladder anlage. Its position corresponds to that of the gall-bladder in other mammals and to the thickening previously described by Helly. The arrangement of the cells is distinct but not striking. The structure seems to be present in another embryo of the same age (Wistar Inst. Coll. No. 15362) which is cut in an oblique plane but only in sagittal sections would it be possible to be sure of it. Lewis and Thyng ('08) have observed well-defined pockets resembling those of intestinal diverticula in the hepatic anlagen of pig embryos, but none were found in specimens under 7.8 mm. in length. This specimen just de-

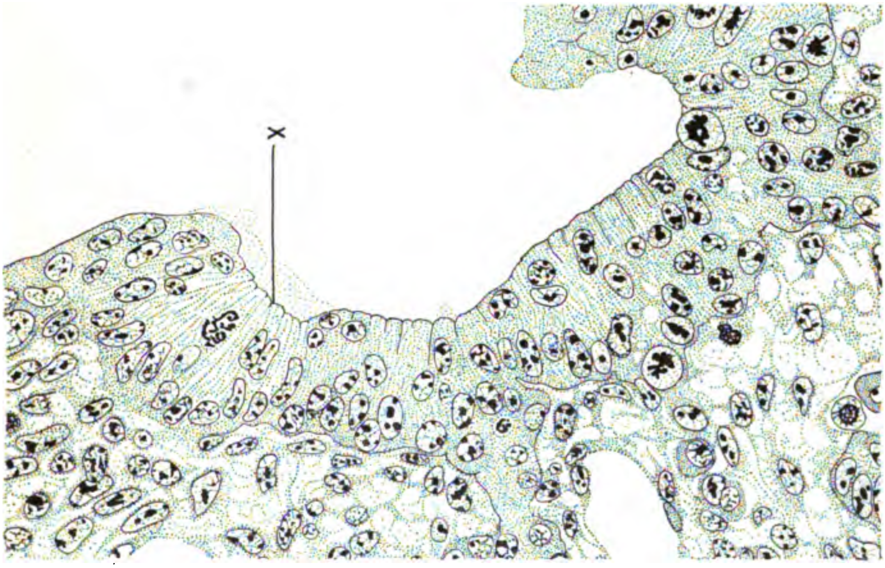

Fig. 10 Sagittal section of the hepatic pouch of an albino rat embryo 3.36 mm. long, 12 days (Wistar Inst. Coll. No. 15363). × 400. *X*, possible cystic anlage.

scribed is of so much earlier a stage that I do not think the two structures can be regarded as identical. I have found no diverticula, such as are described by Lewis and Thyng in other mammals, in the bile-ducts of later rat embryos. Debeyre ('04) has described numerous pancreatic tubules arising directly from the ductus choledochus in rat embryos, but these do not appear until the fifteenth day, and their position differs from that of the swelling described above.

Older embryos examined show no trace of a gall-bladder. In embryos 5.4 mm. long the posterior part of the hepatic pouch has the form of a short wide duct bifurcated at its distal extremity. The lumen is large, particularly distally, and the cells of the ventral wall have peripherally placed nuclei but there is no cystic anlage. Other embryos (11, 13 and 18 mm. in length respectively) also show no trace of a gall-bladder. In all of these specimens the lumina of the hepatic and common bile-ducts are of unusual size.

SUMMARY AND DISCUSSION

The history of the gall-bladder is quite different in each of the three forms under discussion. In Petromyzon a complete biliary apparatus is formed and persists throughout the larval or Ammocoetes stage. At the time of the transformation of the larval to the adult form there is a total degeneration of both gall-bladder and ducts. In the pigeon the gall-bladder is developed apparently in a perfectly normal way, and later, in the majority of cases at least, is completely lost. The duct to which it is attached, persists and grows to some size. This case is complicated, however, by the presence of a larger anterior hepatic duct which opens independently into the duodenum and is never associated with the gall-bladder. In the rat there is at most but a trace of a cystic anlage in very early stages and this soon disappears. I have observed no common factors which would account for the absence of the gall-bladder in all of these examples. In the rat it is possible that the rapid and early reduction in size of the yolk-stalk which leaves the foregut wall quite short antero-posteriorly may be a factor in inhibiting the development of the gall-bladder. In mammals generally, as compared with most lower forms, we find a distinct shortening of the liver anlage and a tendency towards consolidation of the cystic and hepatic portions. This condition is well exemplified in Bos taurus as shown by Pensa's ('13) recent work. The rat may be but an extreme example of this consolidation due to the conditions above mentioned.

The occasional absence of the gall-bladder in man is not explained by these observations. Barring the cases of secondary obliteration of the bile-ducts, it is possible that there are in man two types of inhibition of the development of the biliary apparatus. In one the entire pars cystica of the liver anlage is suppressed. This type would be represented by the rare cases in which both gall-bladder and bile-ducts are absent. In the second only the gall-bladder fundament is lost either early or late in development and this type would be represented by the more common anomaly of absence of the bladder only.

The large size of the bile-ducts is noticeable in both pigeon and rat embryos. This seems to be a compensation for the absence of the usual bile reservoir in the form of a definite sac. A general dilation of the bile-ducts or their local expansion into chambers capable of containing a considerable amount of bile is often but not always found in animals regularly lacking a gall-bladder. This is frequently true in cases of congenital absence of the gall-bladder in the human subject. Dilation of the bile ducts after cholecystectomy is also a common observation.

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GROWTH OF THE BODY AND OF THE VARIOUS ORGANS OF YOUNG ALBINO RATS AFTER INANITION FOR VARIOUS PERIODS

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GROWTH OF THE BODY AND OF THE VARIOUS ORGANS OF YOUNG ALBINO RATS AFTER INANITION FOR VARIOUS PERIODS.

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(Five Tables and Four Charts.)

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Recent investigators have found that the capacity for growth is not lost in young albino rats held at nearly constant body weight for considerable periods, although important changes have taken place in the various organs. When animals thus stunted are generously fed, one of three results might be expected concerning their recovery: (1) There might be a complete recovery in the weight of the body as a whole, with normal proportions of the individual organs and parts. (2) Since the growth impulse and the power of maintenance varies considerably in the different organs and parts of the body, one might expect certain individual organs to show lingering effects of stunting. (3) If the stunting were sufficiently severe to lower the final adult body weight, then the different organs and parts might be either similarly or dissimilarly affected. Thus in the first case, an adult of normal size and proportions would be obtained; in the second case, an adult of normal size but abnormal proportions; in the third case, a dwarf of either normal or abnormal proportions.

Numerous observations are recorded in the literature on the recovery of the body weight as a whole in different animals upon refeeding after various periods of growth suppression; but very few observations have been made upon the individual organs and parts. A more complete and thorough study of this question seemed desirable, and therefore the present investigation was undertaken. This opportunity is taken to express my indebtedness to Dr. C. M. Jackson for valuable aid and direction.

Material and Methods.

For the present experiments eight litters of albino rats (*Mus norvegicus albinus*) were used (Table I.), which included twenty males and twenty-five females, a total of forty-five.

The experiments began when the rats were three weeks of age (time of weaning). From most of the litters, at the beginning of the experiment, one rat of each sex was selected to serve as an (initial) control, the sex being identified by the method of Jackson ('12). Of the nine controls thus selected, two were killed at sixteen weeks of age, and four at about one year; while three were well fed until thirty-nine weeks of age, at which time the experiment for which they were the controls (alternate fasting and refeeding) was discontinued.

In addition to the direct controls, the observations by Jackson and Lowrey ('12), Jackson ('15) and the Wistar norm tables of

Donaldson ('15) were of great value for comparison. In referring to Donaldson's norm throughout the paper, comparison is always made with the weight in animals of corresponding body length, according to the method recommended by Donaldson ('15).

Of the rats subjected to inanition, eighteen were held at nearly constant body weight from three to twelve weeks of age, and killed after being refed one half week (2 rats), one week (5 rats), two weeks (5 rats), or four weeks (6 rats). In addition, two rats of this series were killed at the end of the maintenance period from three to twelve weeks of age. Thirteen rats were refed until adult (about 1 year old) after being held at constant body weight for various periods: from the age of three weeks to age of four weeks (4 rats), from three to six weeks (4 rats), and from three to ten weeks (5 rats). The test rats of litters S23 and S24 were held repeatedly at constant body weight for short periods during the first two or three months, and during the intervening times were amply fed. Later a different plan was adopted, the rats of these two litters being alternately severely starved and refed.

The rats were kept in ordinary wire cages provied with wire net bottoms, which permitted feces and other waste materials to drop into the box base below. It is necessary to use this type of cage to prevent the underfed rats from eating their feces. The cages were kept as clean as possible, and the rats remained healthy with the exception of some lung infection, especially among the older rats.

The temperature of the room in which the control rats were kept remained usually at about 21° C. (70° F.), but occasionally it dropped as low as 16° C. (56° F.). Inasmuch as underfed rats are very susceptible to cold (even a slight chilling being sometimes fatal), a part of them, litters St10, St11, St12, were kept in a separate room where the temperature ranged constantly between 27° and 32° C. (80° and 90° F.). The reduced power of the underfed rats to resist cold is probably due to the exhaustion of reserve material in the body, which ordinarily would be oxidized to maintain the normal temperature.

In spite of keeping the test rats very warm, it was found very difficult to hold them strictly at constant body weight for more than thirteen or fourteen weeks, and keep them alive; so as a rule they were permitted to increase slightly in body weight after that time. Aron ('11) and Jackson ('15) similarly found it increasingly difficult to hold animals at constant body weight as the experiment progressed.

Individual weight records were kept, the individuals rats being identified by staining the integument with an aqueous solution of picric acid. The weight of the rats was always recorded immediately before feeding. The test rats were weighed daily; whereas the controls were weighed at gradually increasing intervals (about once a week after reaching 200 grams body weight).

Previous to reaching sexual maturity the control males and females were usually separated in order to prevent pregnancy. In the case of the stunted rats it was found unnecessary to separate the sexes while being underfed, for in no case did a pregnancy result, although of litter No. S8 the control female and also those rats refed after one and three weeks of maintenance each bore one litter.

All the rats were fed on whole wheat (Graham) bread soaked in whole milk. The control rats were given an abundant amount of food, whereas the test rats received during the underfeeding just that amount of food required to hold them at nearly constant body weight. It was observed that the test rats did not eat their entire maintenance ration immediately until after about two weeks of underfeeding. Aron ('11) noted that at first it was necessary to feed a dog its maintenance ration in two portions, but after a short time it was able to eat its entire allowance at a single feeding. Water in abundance was given to all the animals.

It was found, as was observed by Jackson ('15), that the rats are for a time held at constant body weight upon a gradually diminishing ration. It was further observed, however, that after about fifty days of maintenance there is no further decrease in the necessary amount of food. In one litter, the average amount was found to remain practically constant from the 60th to the 120th day of the experiment.

After being held at maintenance, the test rats were generously fed for different periods as indicated in Table I. At the end of the experiment they were killed and autopsied according to the method used by Jackson ('15), with a few modifications.

TABLE I.

RECORD OF LITTERS USED.

Litter No. and Source.[1]	Number and Sex of		Number and Sex of Rats with Character of Experiment.	Rats Killed at Age of:
	Controls.	Test Rats.		
S8(W)[1] ..	1 M.	3 M.	1 M.–1 F. maint. 3 to 4 wks. of age.	
	1 F.	3 F.	1 M.–1 F. maint. 3 to 6 wks. of age.	
			1 M.–1 F. maint. 3 to 10 wks. of age.	52 weeks
S9(W)...		3 M.	1 M.–1 F. maint. 3 to 4 wks. of age.	
	1 F.	3 F.	1 M.–1 F. maint. 3 to 6 wks. of age.	48 weeks
			1 M.–1 F. maint. 3 to 10 wks. of age.	
S14(W) ..	1 M.	1 M.	1 M. maint. 3 to 10 wks. of age.	63 weeks
S23(W)..	2 M.	2 M.	Alternating short periods of fasting and ample feeding.	——
S24(W)..	1 M.	1 M.	Alternating short periods of fasting and ample feeding.	——
St 10....	1 F.[2]	1 M.	1 F. maint. 3 to 12 wks. of age.	12 weeks
		5 F.	1 F. maint. 3 to 12 wks. of age.	12½ weeks
			1 F. maint. 3 to 12 wks. of age.	13 weeks
			1 M. maint. 3 to 12 wks. of age.	14 weeks
			2 F. maint. 3 to 12 wks. of age.	16 weeks
St 11....		1 M.	1 M.–2 F. maint. 3 to 12 wks. of age.	13 weeks
		6 F.	2 F. maint. 3 to 12 wks. of age.	14 weeks
			2 F. maint. 3 to 12 wks. of age.	16 weeks
St 12....	1 F.[2]	3 M.	1 M. maint. 3 to 12 wks. of age.	12 weeks
		4 F.	1 M. maint. 3 to 12 wks. of age.	12½ weeks
			1 F. maint. 3 to 12 wks. of age.	13 weeks
			2 F. maint. 3 to 12 wks. of age.	14 weeks
			1 M.–1 F. maint. 3 to 12 wks. of age.	16 weeks
Total....	5 M. 4 F.	15 M. 21 F.		

For economy of space, only the average data are indicated in most cases in this paper. A copy of the cards containing the individual data will be filed in the Wistar Institute of Anatomy (Philadelphia) however, where they will be accessible to anyone interested.

On account of the relatively small number of observations, and the known variability of the various organs, the data are inconclusive in some cases. They are, however, sufficient to show clearly some of the more obvious and important changes in the rats refed after various periods of maintenance.

An abstract of the present paper has been published in the

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[1] *W* = litters from Wistar stock; the others are from Minnesota stock.

[2] Controls in litters St 10 and St 12 killed at 16 weeks of age.

Proceedings of the American Association of Anatomists, New Haven meeting, Dec., 1915 (Stewart, '16).

ALTERNATE FASTING AND REFEEDING EXPERIMENTS.

The two litters utilized for the alternate fasting and refeeding experiments included six rats (Table II.), of which three served as the test animals, and three as the controls. The former were frequently held at constant body weight for short periods (3-4 days), and abundantly fed during the intervening time. With each repeated fast the body weight was held constant at a progressively higher level.

TABLE II.

THE GAIN IN BODY WEIGHT IN MALE RATS REFED AFTER BEING HELD AT A CONSTANT BODY WEIGHT FOR SHORT REPEATED PERIODS, AS COMPARED WITH THE CONTROLS.

Litter No.	Rat No.	Total No. of Days the Rats Were Held at Maintenance.	No. of Days the Rats Increased in Body Weight.	Initial Wt. of Rats in Grams.	Final Wt. of Rats in Grams.	Total Gain in Grams.	Daily Average Gain in Grams.
S23	95[1]	0	143	22.4	276.0	253.6	1.77
S23	96[1]	0	143	24.1	218.0	193.9	1.36
S23	97	42	101	22.8	246.0	223.2	2.21
S23	98	42	101	22.1	247.0	224.9	2.22
S24	99[1]	0	121	20.8	254.0	233.2	1.93
S24	100	40	71	19.0	245.0	226.0	3.18
	Average for controls			22.4	249.3	226.9	1.69
	Average for test rats			21.3	246.0	224.7	2.54

For each litter the average daily increase in body weight (Table II.), when growth was permitted was somewhat higher in the test rats than in the controls. Thus the average final weight of the controls, 249.3 grams, was nearly reached by the test rats (average 246.0 grams), although the latter had a much shorter period of actual growth.

On the whole, therefore, the data show the growth of the test rats after short periods of maintenance to be unusually rapid, so they were able to overtake the controls. Seland ('88), however, noted that rabbits and chicks enduring alternate short periods of fasting and generous feeding, became even heavier than the controls. Apparently he obtained a stimulation of growth which enabled the test animals to exceed the controls.

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[1] Controls.

Since this difference in degree of stimulation might possibly be due to the fact that Seland's animals suffered a more severe starvation than mine, it was decided to starve my rats more severely than had previously been done. The test rats of litter S23 were therefore subjected to starvation for three days, and the test rats of litter S24 for four days. They were then abundantly fed for 15 and 20 days respectively, but still failed to exceed the controls in body weight, and showed no tendency to do so. Noè ('00) noted little or no over-compensation in body weight in rats refed after repeated periods of starvation.

TABLE III.

THE AVERAGE DAILY LOSS IN GRAMS IN MALE ALBINO RATS FOLLOWING REPEATED SEVERE STARVATION.

Litter No.	Rat No.	No. of Fast.	Days Fasted.	Wt. Before Fast, G.	Wt. at End of Fast, G.	Loss in Grams.	Loss, Per Cent.	Average Daily Loss, G.
S24	100	First	7	269.5	202.6	67.5	25.0	9.6
S24	100	Second	7	269.0	201.8	67.8	25.1	9.6
S23	97	First	6	240.0	177.5	62.5	26.0	10.4
S23	97	Second	7	241.5	177.5	64.0	26.5	9.1
S23	98	First	7	254.8	191.0	63.8	25.0	9.1
S23	98	Second	8	259.5	192.0	67.5	26.0	8.4

Later the test rats of these two litters (S23 and S24) were used to investigate another point. Kahan ('85) observed that in pigeons the daily average loss in weight increased with each repeated fast involving a loss of 30 to 45 per cent. of the initial body weight. My data (Table III.) show practically no tendency for the daily loss in body weight to increase in rats in repeated severe starvation periods. In fact the average loss for rats Nos. 97 and 98 was slightly less during the second fast than during the first, which suggests that possibly their power to resist starvation had been increased. The difference between my results and those obtained by Kahan may be due to the fact that his animals were more severely starved than mine. It is also probable that different species, as well as different individuals of the same species, may react differently in this respect.

Following the periods of severe starvation the amount of food eaten daily by the rats above mentioned, and their resultant weight, were carefully observed. During the first four days of refeeding, the body weight of the rats increased from an average of 190.2 grams (range 177.5–202.0), to an average of 227.8 grams (209.0–246.0). The average amount of food eaten during the four days of refeeding was 247.7 grams (195–304), which produced an actual average increase of 37.6 grams (31.5–44.0). Thus on the average 15.1 per cent. (13.4–16.6 per cent.) of the ingested food was applied toward increment of body weight (not taking into account the weight of ingested water and salts). In this respect my results are markedly different from those obtained by Morgulis ('11), who found the increase in body weight of starved salamanders following refeeding might even exceed the weight of the ingested food. The absorption of water, however, is doubtless much greater in the case of the salamanders, which probably accounts for the difference.

To summarize, the fasting and refeeding experiments yielded the following results: (1) The daily average gain in weight was greater in the test rats on generous feeding following short periods of fasting than in the controls. The test rats were thus able to overtake (but not to exceed) the controls. (2) The average daily loss in weight did not increase on suffering a second period of starvation, causing a loss of 25 per cent. of the initial body weight. (3) The gain in weight following the severe fasts did not exceed 16.6 per cent. of the weight of the ingested food. (4) So far as body weight is concerned, the rats recovered completely on refeeding after having lost 25 per cent. of their initial body weight.

REFEEDING AFTER VARIOUS PERIODS OF MAINTENANCE.

1. *Growth in Body Weight.*

The average absolute increase in body weight of the test rats refed after the various periods of maintenance, and of the full-fed controls of litters S8, S9, and S14, is represented by the growth curves in charts *A* and *B*. The curves show that the growth of the stunted rats on generous feeding was considerably higher for some time than the normal for the (younger) controls of the same body weight, which enabled the test rats to overtake the controls before the end of the normal growth period.

The unusually rapid growth of the test animals is more strikingly shown in charts *C* and *D*, in which the growth curves of the rats refed after seven weeks of maintenance are superimposed upon the growth curves of the normal rats, so that their starting points coincide. The curves thus constructed show an initial rapid divergence due to the more rapid growth of the test rats during the first few weeks of refeeding. Thus the stunted rats

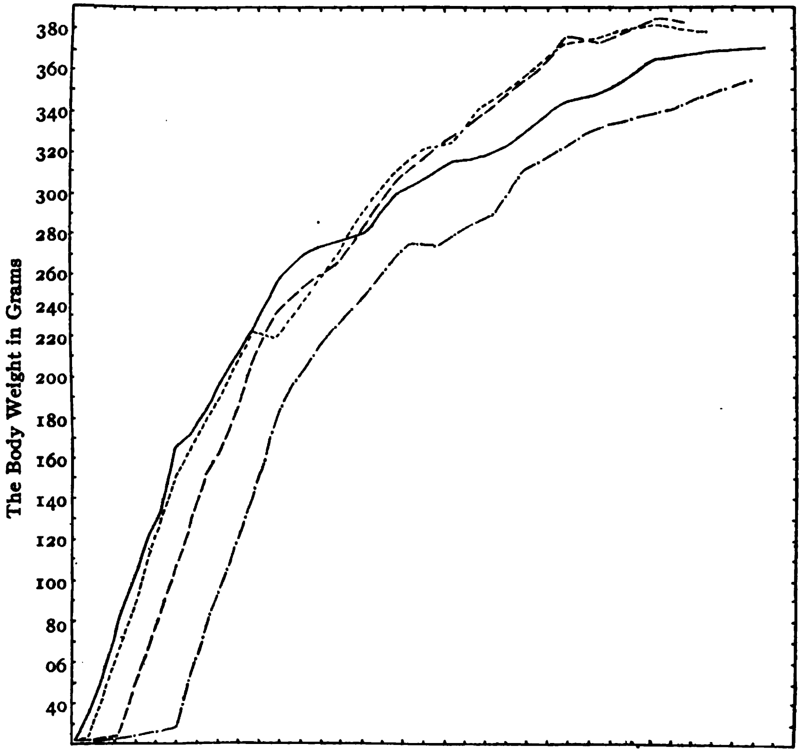

CHART *A*. Males. Chart showing the average absolute increase in weight of the control male rats from the age of three weeks, and also of the test males refed after various periods of maintenance. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. - - - Rats refed after one week of maintenance. – – – Rats refed after three weeks of maintenance. — · — · — · Rats refed after seven weeks of maintenance.

reach the same ultimate body weight as the controls, but in a much shorter period of actual growth. The results therefore agree with those previously stated for the rats subjected to repeated short periods of alternate fasting and refeeding.

This phenomenon of rapid growth following periods of suppression has been observed in various animals by Schapiro ('05) (cat), Hatai ('07) (rat), Miss Springer ('09) (salamander), Morgulis ('11) (salamander), Schloss ('11) and Boas ('12) (human), Miss Ferry ('13) (rat), Osborne and Mendel ('15 and '16) (rat), and others. Osborne and Mendel find that after periods of suppression by various methods the growth upon refeeding appears even more rapid than King's ('15) normal for younger rats of the same size. Although no direct controls were observed their results are very striking.

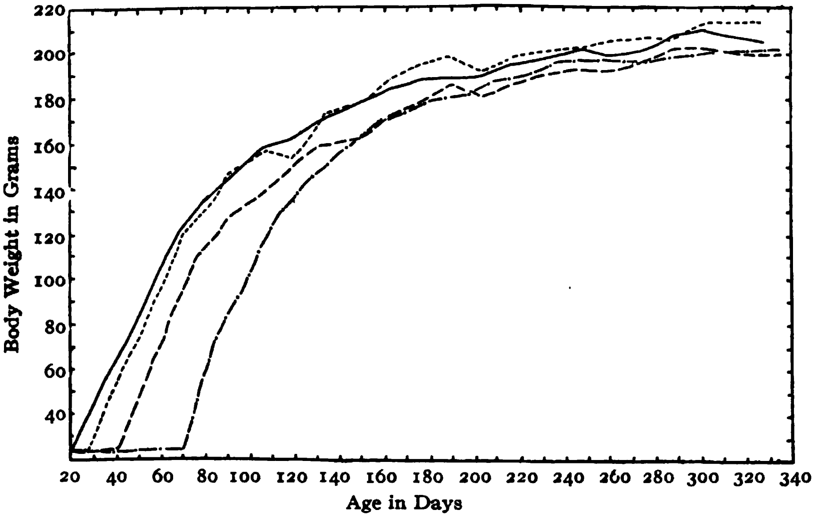

CHART *B*. Females. Chart showing the average absolute increase in weight of the control female rats from the age of three weeks, and also of the test females refed after various periods of maintenance. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. - - - Rats refed after one week of maintenance. — — — Rats refed after three weeks of maintenance. — · — · — · Rats refed after seven weeks of maintenance.

If we accept Minot's ('08) theory that the relative abundance of nuclear material in embryonic cells accounts in part for the greater intensity of embryonic growth, we may in part account for the rapid growth following inanition upon the same principle, the changed nucleus-plasma relation. For it is well known that during inanition the cell nucleus becomes relatively large, the loss of substance being greater in the cytoplasm. Thus inanition tends to reduce the body cells to an embryonic condition, as found by Child ('15) in extensive experiments upon Planarians. It is also possible that the accelerated growth following periods

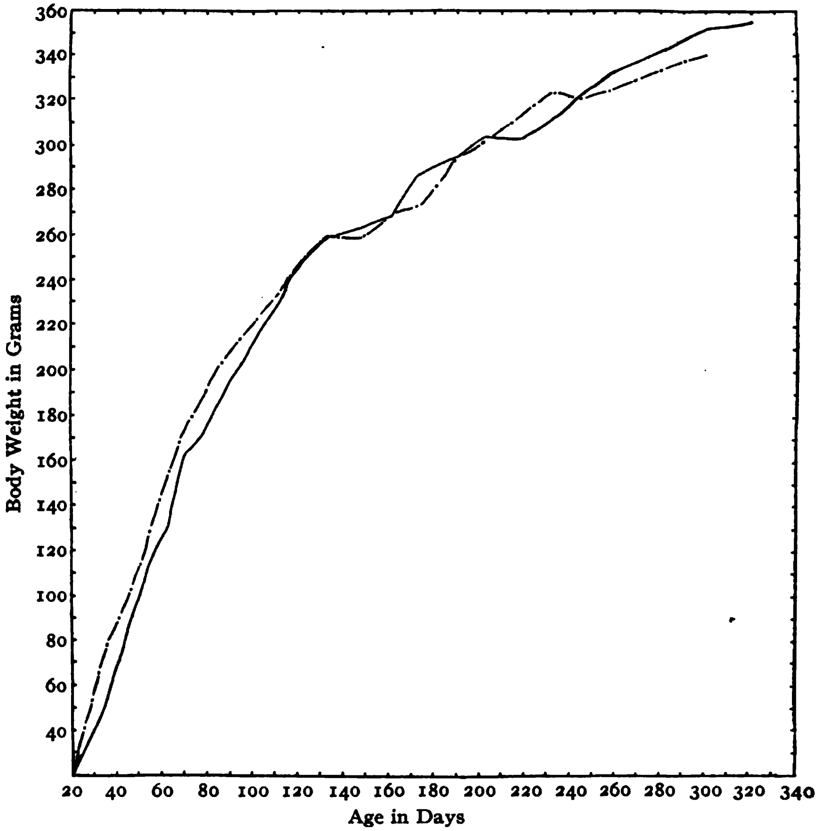

CHART *C*. Males. Chart showing the curve of growth of the test male rats refed after maintenance from three to ten weeks of age, superimposed upon that of the control males, so that the starting points coincide. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9 and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. —·—·—· Rats refed after seven weeks of maintenance.

of suppression is due to specific histological changes in the ductless glands, as suggested by Osborne and Mendel ('16).

In part, however, especially in the first few days of refeeding, the apparent increase in body weight is due to increase in contents of the alimentary canal (and possibly also in the circulating media of the body), which represent increase in gross body weight, but not actual growth of the tissue-cells.

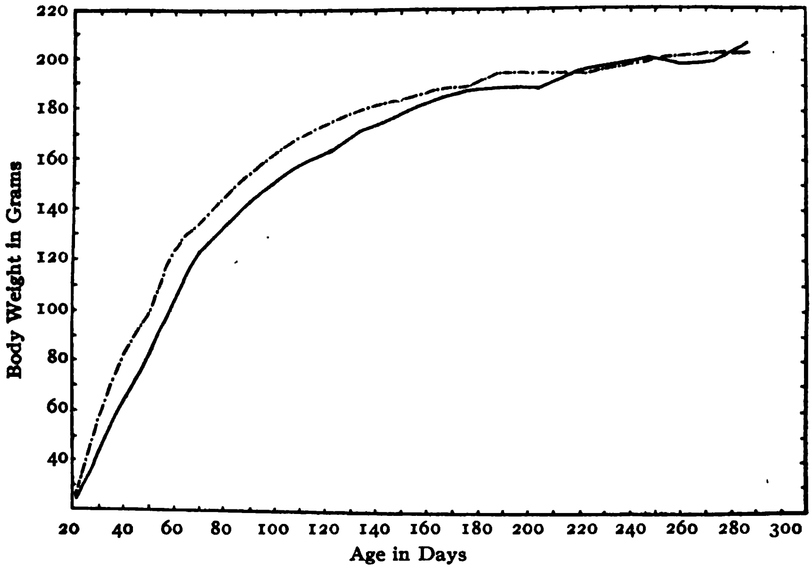

CHART *D*. Females. Chart showing the curve of growth of the test females refed after maintenance from three to ten weeks of age, superimposed upon that of the control females, so that the starting points coincide. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. — · — · — · Rats refed after seven weeks of maintenance.

The rapid growth of the test rats which enabled them to overtake the controls, however, did not persist beyond the point when the normal adult weight was reached. This is evident from the fact that the average adult body weight of the refed animals (Table III.) was not greatly different from that of the controls. Although the controls averaged slightly higher than the test rats of the same sex, the differences are too small to be considered significant. The final weight of the males refed after seven weeks of maintenance averaged considerably below that of the controls, but this was probably due to chance variation, and does not indicate an actual stunting of the test rats. The females show complete recovery.

Aron ('14) noted no permanent stunting effects on the body weight in young rats underfed less than 150 days. Osborne and Mendel ('15, '16) have likewise observed attainment of normal adult body weight after suppression of growth during a period equal to or exceeding the normal growth period. One female (2033) stunted by feeding on a limited quantity of food after the age of 513 days increased from 59 grams to 222 grams in body weight. Osborne and Mendel ('16) in their rats refed after extended periods of growth suppression note a marked tendency even to exceed the normal ultimate body weight observed by King ('15).

In my experiments, the inanition period was begun in rats at an earlier age (3 weeks) than in the investigations just mentioned. Even in these very young rats, however, the recovery in body weight is usually complete, upon refeeding after maintenance periods of one to seven weeks.

The experiments of Brüning ('14), however, indicate that stunting produced by subjecting newborn rats to repeated periods of fasting during the normal nursing period may persist, at least until fifty-four days of age, the test animals usually showing no tendency toward compensatory overgrowth when placed upon an artificial mixed diet. Whether or not complete recovery might occur later was not determined.

2. *Ratio of Tail Length to Body Length* (*Table IV.*).

Jackson ('15) noted that in young albino rats held at constant body weight, the ratio of the tail length to the body length increases from an average of .66 (normal) at three weeks to .84 at ten weeks. In the two rats killed at the end of nine weeks of maintenance the tail-ratio (Table IV.) was .93 and .89, which shows that in my rats the tail likewise became relatively long during the inanition period.

At the end of each period of refeeding (Table IV.), the average tail-ratio, in the test rats (with two exceptions) ranged between .80 and .84, which is a little below the normal for rats of corre-

TABLE IV.

AVERAGE GROSS AND NET BODY WEIGHT, TAIL RATIO, AND RELATIVE WEIGHT (PERCENTAGE OF NET BODY WEIGHT) OF HEAD, TRUNK, EXTREMITIES, INTEGUMENT, SKELETON, MUSCULATURE, VISCERA AND "REMAINDER."

	Rats Held at Maintenance from 3 to 12 Weeks of Age and Autopsied.										Con-trols.	Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year.		Controls Age About One Year.	
	Immediately.		After Refeeding ½ Week.		After Refeeding 1 Week.		After Refeeding 2 Weeks.		After Refeeding 4 Weeks.		Age 16 Weeks.				
No. of Rats and Sex	1 M.	1 F.	1 M.	1 F.	1 M.	4 F.	1 M.	4 F.	1 M.	5 F.	2 F.	7 M.	6 F.	2 M.	2 F.
Gross body weight, g.	45.0	25.5	48.5	40.5	62.2	57.0	91.0	75.3	125.2	113.2	127.7	347.9	208.8	351.5	210.7
Net body weight, g.	41.2	24.3	44.9	36.6	58.3	51.5	86.3	69.4	117.4	105.0	122.6	337.8	200.4	341.6	203.2
Ratio of tail to body length	.93	.89	.80	.81	.82	.83	.74	.80	.76	.84	.85	.80	.89	.79	.88
Head %	17.7	22.2	16.7	18.0	14.1	15.1	11.9	13.2	10.3	11.2	10.9	7.29	8.6	7.4	8.2
Upper extrem. %	8.5	9.1	7.8	8.7	7.2	7.6	7.2	7.4	6.2	6.7	5.9	5.5	6.0	5.5	5.8
Lower extrem. %	15.8	17.7	16.9	15.6	16.3	16.2	17.3	17.1	16.1	16.4	18.1	15.1	15.9	15.6	15.0
Trunk %	58.0	51.0	58.6	57.7	62.4	61.2	63.5	62.5	67.9	65.8	65.2	72.2	69.4	71.2	70.8
Integument %	15.5	16.5	15.4	14.2	14.8	17.4	20.7	20.5	18.8	19.9	18.3	20.7	17.9	18.7	17.5
Lig. skeleton %	17.2	19.8	16.5	15.3	12.9	14.8	12.2	12.4	10.4	12.2	11.5	7.9	9.3	8.1	8.9
Wet cart. skeleton %	15.8	18.1	14.3	12.6	10.3	10.8	7.7	8.9	5.5	9.1	8.5	6.0	7.1	6.9	6.8
Dry cart. skeleton %	5.96	5.24	5.70	4.35	4.05	4.44	3.17	3.63	3.39	4.07	3.99	3.62	4.18	3.69	3.97
Musculature %	36.2	38.3	35.6	33.1	32.3	31.4	34.1	32.1	41.2	40.7	39.6	42.8	43.9	42.3	42.8
Visceral group %	19.0	20.1	22.1	20.9	19.4	20.2	17.7	17.7	19.5	17.0	14.2	11.5	13.0	11.1	12.3
"Remainder" %	12.1	5.4	10.5	16.4	20.7	16.3	15.3	17.4	10.4	10.4	16.6	17.3	16.4	19.7	18.4

sponding body weight. In the females refed four weeks the ratio (.84) was practically identical with that in the controls (.85). For the male rat of this group the value was exceptionally low (.76), as was also true of the male refed two weeks. In the adult rats the average tail-ratio was practically normal in the test males and females, as compared with the controls of the same sex.

In general, therefore, it appears that the tail and body assume the normal proportions (for corresponding body weight) in the test rats during the first week of refeeding, and remain normal at all subsequent periods. The exceptional cases are probably due to normal variability.

3. *Head* (*Table IV.*).

According to Jackson ('15), the head normally forms an average of 22.5 per cent. of the body, the average net body weight being 21.2 grams. In the female rat killed after being kept at constant body weight (24.3 grams net) from three to twelve weeks of age, the head (Table IV.) formed 22.2 per cent. of the net body weight. In the male rat of this group the relative weight of the head (17.7 per cent.) was unusually low, which was due to the body weight being unusually high. It is probable that the weight of the head remained practically unchanged during maintenance, as noted by Jackson ('15).

On refeeding (as also during normal growth) the relative weight of the head gradually decreases, reaching an average of 15.1 per cent. in the females refed one week, whose net body weight averaged 51.5 grams. This is nearly identical with the relative weight (15.2 per cent.) of the head observed by Jackson ('13) for normal rats of practically the same body weight (50 grams). The relative weight of the head in the rats refed four weeks was practically the same as in the controls of the same age (Table IV.). In the adult rats the head was normal in the test animals as compared with the controls. On the whole, then, the head appears to have remained practically normal in relative weight throughout the period of refeeding.

4. *Extremities and Trunk* (*Table IV.*).

The relative weight of the upper extremities 8.5 and 9.1 per cent. (Table IV.) in the two rats killed at the end of nine weeks of maintenance, compares closely with the relative weight (9.3 per cent.) obtained by Jackson and Lowrey ('12) for the normal three week rat. According to Jackson ('15), there is apparently a slight decrease in the relative weight of the fore-limbs in the test rats from 9.3 per cent. to an average of 8.8 per cent., which, however, might be due to accidental variation.

On refeeding, the relative weight of the forelimbs gradually decreased, reaching an average of 7.4 per cent. (sexes combined) at the end of the second week, with average net body weight of 72.8 grams. Jackson and Lowrey ('12) found the upper extremities to form on the average 6.7 per cent. of the body in rats weighing 79.2 grams net. The weight then in my rats refed two weeks, although slightly higher, is therefore nearly normal. At the end of four weeks of refeeding the upper extremities were also slightly heavier in the test rats than in the controls; but in the adult rats the relative weight was practically normal in the refed individuals, as compared with the controls.

In general, therefore, the forelimbs appear practically normal throughout the various refeeding periods, though perhaps relatively somewhat heavy at the end of two and four weeks of refeeding.

The lower extremities (Table IV.) formed 15.8 and 17.7 per cent. of the body in the two rats killed after maintenance for nine weeks. This is practically identical with the normal at three weeks of age (15.7 per cent.) found by Jackson ('15), who also found no distinct change in the weights of the extremities in young rats held at maintenance for considerable periods.

On refeeding, the relative weight of the lower extremities at the end of the second week averaged 17.1 per cent. of the average net body weight (72.8 grams) which is slightly higher than the value (14.9 per cent.) observed by Jackson and Lowrey for rats averaging 79.2 grams. At the end of four weeks of refeeding the lower extremities were relatively lighter in the test rats than in the controls, whereas in the adult the weights were practically identical in the refed and control animals.

The relative weight of the trunk (Table IV.) averaged 54.5 per cent. in the rats killed at the end of the maintenance period, which corresponds closely with the weight (54.1 per cent.) observed by Jackson for the trunk in normal rats at three weeks.

At the end of two weeks of refeeding the trunk formed an average of 62.7 per cent. (sexes combined) in the rats weighing 72.8 grams, as compared with 63.2 per cent. noted by Jackson and Lowrey in normal rats at 79.2 grams net body weight.

In the rats refed four weeks, and also in the adult test rats, the relative weight of the trunk was practically normal as compared with the controls.

The results concerning the different parts of the body therefore fail to show any decided deviation from the normal proportions throughout refeeding after various periods of maintenance.

5. *Integument* (*Table IV.*).

Jackson ('15) observed that the relative weight of the integument in rats held at maintenance from three to ten weeks of age, decreased from an average of 21.9 per cent. to 14.5 per cent. of the net body weight. The data for my rats show likewise a low relative weight for the integument (average of 16.0 per cent.) in rats held at maintenance for nine weeks.

At the end of the first half week, and first week of refeeding the relative weight of the integument was still unusually low in the test rats, although an increase is apparent in the females refed one week. During the second week of refeeding the integument rapidly recovered the loss suffered during inanition, forming over 20 per cent. of the body. This average is close to the normal at corresponding body weight found by Jackson and Lowrey ('12). In the rats refed four weeks, and also in the adult test animals the integument was relatively slightly heavier than in the controls of the same age. The difference, however, is probably insignificant.

It therefore appears that on refeeding the integument rapidly recovers the loss suffered during maintenance, reaching the normal proportions within the first two weeks, and remains practically normal at all subsequent periods.

6. *Skeleton* (*Table IV.*).

Three weighings were taken of the skeleton prepared as described by Jackson ('15), who found that the skeleton increases greatly in weight during maintenance. The high relative weight of the ligamentous skeleton (17.2–19.8 per cent.) in my two rats killed after nine weeks of maintenance as compared with Jackson's normal at three weeks (15.7 per cent.) is in agreement with this conclusion.

On refeeding, the relative weight of the ligamentous skeleton gradually decreases, reaching an average (in the females) of 12.4 per cent. at the end of the second week. This is slightly lower than the percentage weight (14.0) obtained by Jackson and Lowrey for the ligamentous skeleton in normal rats of about the same body weight. It appears then that the skeleton had decreased in relative weight during the first two weeks of refeeding sufficiently to reach the normal proportions. In the rats refed four weeks, and also in the adult test rats, the relative weights of the ligamentous skeleton were practically normal as compared with the controls.

The data (Table IV.) show that a marked increase occurred also in the moist cartilaginous skeleton in the test rats during maintenance, as is evident upon comparison with the relative weight (11.4 per cent.) given by Jackson ('15) for the normal rat at three weeks. During refeeding, the relative weight of the moist cartilaginous skeleton gradually decreased, reaching an average of 11.9 per cent. of the body in the rats refed four weeks, as compared with an average of 11.5 per cent. in the controls. In the adult rats there was very little difference between the test animals and controls.

The dried cartilaginous skeleton was also relatively heavier in the maintenance rats at twelve weeks (5.24–5.96 per cent.) than the normal (3.43 per cent.) found by Jackson for rats at three weeks of age. On refeeding, the relative weight of the dried skeleton has decreased notably by the end of one week (Table IV.), and has probably reached the normal at two weeks. After four weeks' refeeding, and in the adults, the percentage of the dry skeleton is nearly identical in test animals and controls.

The relative amount of dry substance in the cartilaginous skeleton had also increased from 31.4 per cent. (Jackson, '15) for the normal three weeks' rat, to an average of 34.5 per cent. in the rats killed after nine weeks of maintenance. In the rats refed four weeks the dry substance increased to 45.9 per cent. of the moist cartilaginous skeleton, which is fairly close to that found in the control (46.6 per cent.).

It therefore appears that the ligamentous and cartilaginous skeletons reach the normal proportions during the first two weeks of refeeding. This is probably true also for the dry skeleton, although controls are lacking before the fourth week.

7. *Musculature* (*Table IV.*).

In the two rats killed at the end of nine weeks of maintenance the relative weight of the musculature (36.2–38.3 per cent.) is considerably higher than the norm (31.2 per cent.) obtained by Jackson ('15) for the three weeks' rat. This may be due partly to an increase in the musculature during maintenance (a slight increase being found by Jackson). The body weight is above normal in these two rats, however, in which case the musculature would normally be relatively heavier. For the normal rat at 42.4 grams, Jackson found the musculature forming 35.3 per cent., although at 64.4 grams body weight Jackson and Lowrey ('12) found the musculature forming 32.7 per cent.

In my test rats refed one half week (average net weight 40.7 grams) the musculature averaged 34.3 per cent., whereas in the female rats refed two weeks (average net weight 69.4 grams) the musculature formed only 32.1 per cent. of the body. There is evidently considerable individual variation, and it is difficult to make the dissection of the musculature in a uniform manner. In the rats refed four weeks, and also in the adult test animals the percentage weight of the musculature was nearly identical with that of the controls.

From the foregoing it may be concluded that the musculature (which possibly was slightly heavier than the normal for body weight at the end of maintenance), assumed practically the normal proportions during the first week of refeeding and remained normal at all subsequent periods.

8. *Viscera and "Remainder"* (*Table IV.*).

The relative weight of the visceral group (including the abdominal and thoracic viscera, spinal cord, brain and eyeballs) in the rats killed at the end of the fasting period corresponds closely with the weight (20.5 per cent.) found by Jackson ('15) for the normal rat of three weeks. Concerning the visceral group, Jackson noted during maintenance a distinct tendency to increase in weight, which was more marked at six and eight than at ten weeks. The fact that my rats fail to show an increase in the relative weight of the visceral group probably is due to their longer period of stunting, during which the liver especially tends to decrease in weight.

According to Jackson and Lowrey, the relative weight of the visceral group is 21.3 and 20.4 per cent. of the body at three and six weeks of age, the net body weight averaging 24.8 and 64.4 grams respectively. In my rats refed one week and less, the relative weight of the viscera (Table IV.) in all cases formed about 20 per cent. of the body, thus being approximately normal. At the end of two weeks of refeeding however the visceral group appears exceptionally light, the relative weight being 17.7 per cent. as compared with 20.4 per cent. given above for the normal rat of about the same body weight (64.4 grams). After four weeks of refeeding the viscera collectively were relatively heavier in the test rats than in the controls, whereas in the adult rats the weights are nearly identical in the test animals and controls.

In general it therefore appears that the relative weight of the visceral group was about normal during the refeeding. A considerable variation in the weight of the visceral group is not unusual.

The weight of the "remainder" (Table IV.), which includes some small unweighed organs, fat, and body-fluids, was obtained by deducting the weight of the integument, skeleton, musculature, and viscera from the net body weight. The data show considerable variation but it is doubtful whether there is any material change from the normal during refeeding in the test rats.

9. *Brain* (*Table V.*).

The brain shows but little deviation from Donaldson's ('15) Wistar norm for rats of same body length in either the rats killed at the end of maintenance or in those refed for various periods. Such slight fluctuations from the norm as are shown in the table are probably within the range of normal variation. Jackson ('15) noted that there was practically no change in the brain weight of young albino rats held at constant body weight for considerable periods.

It may therefore be concluded that the brain weight is normal in the young rats at the end of the maintenance period, and remains normal throughout the period of refeeding.

10. *Spinal Cord* (*Table V.*).

The weight of the spinal cord in the two rats killed at the end of the maintenance period exceeded Donaldson's norm, 34.3 and 19.9 per cent. This confirms Jackson's ('15) observation that the spinal cord shows a marked growth in young rats held at constant body weight.

On refeeding, this excess weight (as compared with normal for corresponding body length) rapidly disappeared, so that by the end of the first week the weight of the spinal cord exceeds Donaldson's norm only 7.3 and 3.2 per cent. in the 1 male and 4 females respectively. Thus the spinal cord had nearly regained the normal proportion.

At the end of two and four weeks of refeeding the weight of the spinal cord was slightly below the Wistar norm, as was also true of the controls at sixteen weeks of age. This condition therefore appears to be normal for my series of rats and not an experimental modification.

In the test rats (refed after maintenance for various periods) killed at the age of one year the weight of the spinal cord was normal as compared with the controls, although in each case the average weight was 8 to 10 per cent. heavier than Donaldson's norm.

In general therefore the results indicate that the spinal cord, which was relatively heavy at the end of the maintenance period, returned to the normal proportion during the first two weeks of refeeding, and remained normal after that time.

TABLE V.

THE AVERAGE ABSOLUTE WEIGHT OF THE ORGANS IN GRAMS AND THE PERCENTAGE DEVIATION FROM DONALDSON'S ('15) NORM FOR RATS OF CORRESPONDING BODY LENGTH (EXCEPT IN CASE OF THYMUS WHERE AGE IS BASIS OF COMPARISON). FOR CORRESPONDING BODY WEIGHTS AND LENGTHS, SEE TABLE IV.

Organ.	Rats Held at Maintenance from 3 to 12 Weeks of Age and Killed.										Controls.	Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year.		Controls Age About One Year.	
	Immediately.		After Refeeding ½ Week.		After Refeeding 1 Week.		After Refeeding 2 Weeks.		After Refeeding 4 Weeks.		Age 16 Weeks.				
No. and Sex of Rats	1 M.	1 F.	1 M.	1 F.	1 M.	4 F	1 M.	4 F.	1 M	5 F.	2 F.	7 M.	6 F.	2 M.	2 F.
Brain	1.542	1.335	1.479	1.420	1.574	1.472	1.597	1.531	1.703	1.720	1.718	1.974	1.835	1.933	1.819
Dev. %	+5.56	+1.02	+1.23	+2.13	+3.8	−.67	−1.31	+.97	−1.24	+2.74	+.95	−.38	+.65	−3.15	−.69
Sp. cord	.365	.259	.335	.260	.327	.313	.337	.328	.441	.424	.452	.727	.626	.751	.646
Dev. %	+34.3	+19.9	+23.3	+ 4.1	+ 7.3	+ 3.1	− 9.5	− 4.9	− 2.5	− 3.6	− 2.7	+ 8.1	+10.0	+ 7.8	+11.9
Eyeballs	.203	.175	.208	.193	.205	.202	.202	.203	.227	.236	.245	.336	.314	.361	.310
Dev. %	+42.8	+45.5	+46.2	+46.1	+33.4	+33.7	+12.6	+22.0	+ 6.8	+16.2	+15.0	+ .39	+19.3	+1.7	+15.9
Thyroid	.0065	.0098	.0071	.0052	.0072	.0071	.0094	.0083	.0174	.0159	.0186	.0478	.0275	.0424	.0307
Dev. %	−35.2	+25.7	−32.4	−43.5	−40.5	−39.3	−40.9	−40.3	−18.7	−20.1	−13.9	+ 5.1	−10.7	−14.0	− 3.2
Thymus	.0356	.0177	.0502	.0648	.0898	.0769	.242	.180	.428	.383	.214	.086	.071	.051	.076
Dev. %	−87.7	−93.9	−82.6	−77.5	−68.0	−72.6	− 9.0	−32.5	+70.4	+52.5	−14.6	+59.8	+31.3	− 6.4	+41.5
Heart	.223	.130	.279	.206	.343	.304	.488	.411	.583	.580	.627	1.1787	.745	1.060	.744
Dev. %	−15.6	−34.1	+ 5.8	−11.8	+12.6	+ 3.0	+22.9	+17.7	+ 9.8	+17.6	+17.0	+ 3.8	− 2.6	−14.0	− 5.4
Lungs	.313	.213	.361	.336	.378	.376	.579	.530	1.280	1.088	1.085	1.502	1.152	1.155[2]	1.002
Dev. %	−17.1	−27.0	−4.19	− .24	−12.3	−10.0	+ 6.6	+ 7.6	+69.3	+42.0	+44.1	−12.9	+ 2.9	−38.6	−13.1
Liver	2.104	.998	3.66	2.19	3.60	3.27	4.39	4.06	6.57	5.96	5.23	14.18	9.18	11.99	8.05
Dev. %	−37.5	−59.4	+ 8.8	−25.4	− 7.6	−14.0	−12.8	− 9.4	− 2.8	− 5.3	−23.3	+ .95	− 4.3	−21.0	−18.3
Spleen	.124	.055	.204	.177	.416	.319	.555	.383	.424	.472	.597	.979[1]	.710	1.105	.713
Dev. %	−10.7	−44.9	+46.9	+47.5	+154	+107	+144	+98.4	+31.3	+59.9	+82.8	+20.4	+40.7	+23.5	+36.6

[1] One enlarged spleen excluded.

[2] Pathological lungs of one rat excluded.

TABLE V.—*Continued.*

Organ.	Rats Held at Maintenance from 3 to 12 Weeks of Age and Killed.										Controls.	Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year.		Controls Age About One Year.	
	Immediately.		After Refeeding ½ Week.		After Refeeding 1 Week.		After Refeeding 2 Weeks.		After Refeeding 4 Weeks.		Age 16 Weeks.				
No. and Sex of Rats......	1 M.	1 F.	1 M.	1 F.	1 M.	4 F.	1 M.	4 F.	1 M.	5 F.	2 F.	7 M.	6 F.	2 M.	2 F.
Empty al. tract...........	2.23	1.30	2.48	2.33	3.32	3.47	5.35	3.75	8.73	5.78	5.39	11.21	9.41	10.92	9.80
Dev. %..................	−36.7	−48.8	−29.6	−24.4	−19.1	−12.4	+ .20	−20.2	+25.1	−11.5	−16.5	−18.5	− 2.8	−25.7	− 1.2
Suprarenal glands..........	.0146	.0130	.0128	.0118	.0149	.0134	.0180	.0162	.0182	.0271	.0347	.0442	.0431	.0430	.0406
Dev. %..................	+36.0	+16.1	− 9.2	−11.3	− 5.1	−21.2	− 7.2	−20.6	−25.4	−10.2	+ 4.2	− 3.5	−14.1	−12.4	−21.6
Kidneys..................	.464	.375	.600	.442	.615	.571	.853	.715	1.097	1.130	1.254	2.795[3]	1.872	2.553	1.711
Dev. %..................	−10.4	− 4.2	+15.8	− 3.7	+ 2.8	− 1.2	+ 8.4	+ 7.7	+ 1.7	+23.5	+15.4	+1.02	+15.4	− 8.0	+2.3
Testes..................	.175	......	.201	......	.460	......	.534	......	1.185	......	......	2.213[4]	......	2.448	......
Dev. %..................	−49.8	......	−42.2	......	− 7.5	......	−46.1	......	−20.7	......	......	−19.1	......	−14.5	......
Ovaries..................	......	.0072	......	.0069	......	.0126	......	.0143	......	.0440	.0426	......	.0429	......	.0408
Dev. %..................	......	+ 1.4	......	−11.6	......	+43.2	......	+44.5	......	+27.9	− 1.2	......	−11.9	......	−16.6
Hypophysis..............	.0023	.0023	.0028	.0022	.0031	.0030	.0033	.0031	.0056	.0078	.0078	.0117	.0168	.0104	.0181
Dev. %..................	−14.8	+ 9.5	+ 4.7	− 8.3	+ 3.3	0	−13.2	−20.6	+12.0	+13.5	+ 2.6	+10.4	+30.3	− 9.6	+35.1
Pineal..................	.0008	.0008	.0010	.0010	.0009	.00085	.0011	.0010	.0013	.0013	.0014	.0017[5]	.0014	.0015	.0016

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[3] Kidneys of one rat overlooked.

[4] Testis of one rat excluded (pathological).

[5] One pineal body excluded.

11. *Eyeballs* (*Table V.*).

As compared with Donaldson's ('15) norm, the eyeballs were excessively heavy (plus 42.8 and 45.5 per cent.) in the two rats killed at the end of nine weeks of maintenance. The persistent growth of the eyeballs in young rats held at constant body weight was discovered by Jackson ('15). In the rats refed one half, one, and two weeks the eyeballs are still relatively heavy as compared with Donaldson's norm, although the difference has decreased somewhat during these periods. Even in the rats refed four weeks the eyeballs are still somewhat above Donaldson's norm, but are practically normal as compared with my controls.

Likewise in the adult refed rats, as compared with the controls, the eyeballs appear to be normal in weight, although in the females they somewhat exceed the Wistar norm in weight.

The evidence therefore indicates that the eyeballs, which were relatively heavy at the end of the maintenance period, returned to the normal proportion by the end of the fourth week of refeeding, and were normal in the adult test rats.

12. *Thyroid* (*Table V.*).

At the end of the maintenance period the weight of the thyroid in the male rat was 35.2 per cent. below the Wistar norm, while in the female it was 25.7 per cent. above. Jackson ('15) noted that the thyroid suffered a marked loss during maintenance. The exceptionally large weight for the female above noted may be due to experimental error or normal variation. On refeeding, the thyroid remains below Donaldson's Wistar norm, although by the fourth week it is only 18.7 to 20.1 per cent. below. In the controls for this group the weight of the thyroid is also below Donaldson's norm by 13.9 per cent., which deducted from the minus 20.1 per cent. for the test females leaves 6.2 per cent. loss apparently produced by the fasting experiment. In view of the known variability of the thyroid, this difference is too small to be considered significant. We may therefore conclude that the thyroid gland has *probably* recovered its normal weight during the four weeks of refeeding. In the adult rats the fluctuations in the weight of the thyroid gland are likewise well within the limits of normal variability, and show no indications of any persistent effects of the earlier maintenance period.

13. *Thymus* (*Table V*).

The weight of the thymus at the end of the maintenance period was from 87.7 to 93.9 per cent. below Donaldson's norm. This agrees closely with Jackson's ('15) observation of 90 per cent. loss in the weight of the thymus due to "hunger involution" during maintenance in young rats from the age of 3 to 10 weeks. At the end of two weeks of refeeding, the thymus was still somewhat below Donaldson's norm (9.0 to 32.5 per cent.); but at the end of the fourth week it *exceeded* the norm 70.4 and 52.5 per cent. in the male and female test rats respectively, while the controls averaged 14.6 per cent. below. It is therefore evident that the thymus returned to the normal proportion between the second and fourth weeks of refeeding. The weight of the thymus in the test rats refed four weeks was remarkably high, indicating a marked over-compensation of the loss during "hunger involution."

In the adult rats the thymus was relatively heavier in the female controls than in the female test rats, whereas the converse was true of the males. This inconsistency, together with the known variability in the normal weight of the thymus (average coefficient of variation is 34, according to Jackson, '13) makes it doubtful whether the result is due to the experimental conditions or is due merely to normal variation. Further observations will be necessary to determine this question.

The results in general therefore indicate that in the stunted rats, the thymus returns to the normal proportion shortly after the second week of refeeding, and greatly exceeds the normal at the end of the fourth week. The data for rats refed to the adult stage indicate the possibility of a permanent over-compensation in the growth of the thymus upon refeeding after a period of maintenance.

Jonson ('09) subjected a rabbit (age 6 weeks) to inanition for 31 days, during which time the body weight increased from 553 to 655 grams. The animal was then refed for three weeks, at the end of which time the weight of the thymus was found normal as compared with the weight in a control killed at the end of the fasting period.

Salkind ('15) observed that a minimum of at least one week of refeeding was necessary to restore the normal lymphoid structure of the thymus in rats suffering severe starvation for two days.

14. *Heart* (*Table V*).

The heart at the end of the maintenance period was 15.6 and 34.1 per cent. below Donaldson's norm of weight. Jackson ('15), however, noted practically no change in the weight of the heart during maintenance. The discrepancy in my rats may possibly be due either to normal variation or to experimental error.

At the end of the various refeeding periods the heart (with one exception) was heavier than Donaldson's norm, the excess varying from 3.0 to 22.9 per cent. At the end of four weeks of refeeding, the weight of the heart in the test females exceeded the Wistar norm 17.6 per cent. while the controls exceeded it by 17.0 per cent. The heart therefore appears practically normal in weight at sixteen weeks in the refed rats as compared with the controls. This was also true for the adult animals.

In general, therefore, it may be concluded that the heart was probably nearly normal in the test rats throughout refeeding, although it appears relatively heavy (in comparison with Donaldson's norm) during the earlier weeks of refeeding.

15. *Lungs* (*Table V.*).

The weight of the lungs was 17.1 and 27.0 per cent. below the Wistar norm at the end of nine weeks of maintenance, indicating a decrease in size during that period. Jackson ('15) found a loss of about 15 per cent. during seven weeks of maintenance. The lungs were also somewhat below the norm during the first week of refeeding, but somewhat above thereafter. The excess was especially marked in the rats refed four weeks (42.0 to 69.3 per cent.), but the controls also showed an excess weight of 44.1 per cent. This excess might be due to an over-compensation of growth, or merely to variability from other causes (possibly pathological, due to slight pulmonary infection). In the adult rats the lungs were smaller than the norm except in the case of the test females, which were slightly larger. The differences are probably due to individual variation.

The results therefore indicate that the lungs, which lose weight during maintenance, completely recovered within two weeks of refeeding. There is possibly an over-compensatory growth before the end of four weeks, but no indication of such in the animals refed to the adult stage.

16. *Liver* (*Table V.*).

The weight of the liver in the two rats killed at the end of nine weeks of maintenance was 37.5 and 59.4 per cent. below Donaldson's norm for the male and female respectively. (However, Jackson ('13) found the normal liver weight, especially in young rats, considerably below the curve derived from Hatai's formula, upon which Donaldson's norm is based.) Jackson ('15) found the liver to increase in weight in rats held at maintenance from three to six and ten weeks of age, but it decreased in those held for longer periods (from six to thirty-two weeks of age). The low weight of the liver for my rats at the end of the maintenance period was probably due in part to the length of their maintenance period.

During the first four weeks of refeeding the weight of the liver was below Donaldson's norm, except in the male refed one half week. In most instances, however, especially after the first week, the deviations are within the limits of normal variation. In the controls at sixteen weeks, however, the weight of the liver was 23.3 per cent. below the norm, while in the test females of the same age it was but 5.3 per cent. below. Thus when compared with Donaldson's norm, the liver is about normal in the test rats refed four weeks, but when compared with the controls it is relatively heavy.

In the adult test rats the weight of the liver was very close to Donaldson's norm, but very low (—18.3 to —21.0 per cent.) in the controls.

Therefore in comparison with the Wistar norm the liver appears practically normal after the first week of refeeding. As compared with the controls, however, there is some indication of hypertrophy in the liver of the rats refed four weeks, and also in the adult test animals. It is more probable that this apparent increase is due merely to normal variability, which is quite large in the liver.

17. *Spleen* (*Table V.*).

Jackson ('15) noted a considerable decrease in the weight of the spleen in rats held at maintenance from three to eight and ten weeks of age. My data (Table V.) likewise indicate that the spleen is low in weight at the end of nine weeks of underfeeding (minus 10.7 and 44.9 per cent.) when compared with Donaldson's norm.

On refeeding, at all periods the weight of the spleen was much higher than Donaldson's norm, the excess appearing greater at one and two weeks of refeeding, than at later periods. In the controls the spleen likewise exceeded the Wistar norm, 82.8 per cent. at sixteen weeks, and 23.5 and 36.6 per cent. in the adult control males and females respectively. As compared with the controls the spleen was practically normal in the adult test rats.

In this connection it may be noted that, as was pointed out by Jackson ('13), Hatai's formula for the growth of the spleen (upon which Donaldson's norm is based) gives a curve which in general is too low (excepting the earlier stages), because he excluded all "enlarged" spleens from the series, without apparent justification. In most cases, however, the spleens of my refed rats appear considerably heavier even than Jackson's curve. The fact that the weight of the spleen is normally exceedingly variable, as shown by Jackson ('13) must also be kept in mind.

In general therefore the results indicate that upon refeeding the spleen rapidly (within ½ week) recovered the loss suffered during fasting. There is apparently an excessive (over-compensatory) growth of the spleen during the first two weeks of refeeding. At the end of the fourth week, however, and in the adult rats the spleen in the test animals is not above that of the controls.

18. *Stomach and Intestines* (*Table V.*).

The weight of the empty alimentary tract at the end of maintenance was much below the Wistar norm (minus 36.7 to 48.8 per cent.). My data, however, are somewhat above the weights observed by Jackson ('15) for the empty tract (in controls at body weight of 25.1 and 42.4 g.). This would indicate an increase in the weight of the tract during maintenance, in agreement with Jackson ('15).

On refeeding, with the exception of the male refed four weeks, the weight of the empty alimentary tract increased in relative size, but remained below the Wistar norm in most cases. It was higher in the refed rats than in the controls at the age of 16 weeks (end of fourth week of refeeding). In the adult rats the weight of the empty stomach and intestines was practically normal in the test rats as compared with the controls, although below the Wistar norm, especially in the males.

The relative weight of the stomach and intestines including contents (12.6 per cent.) in the two rats killed at the end of nine weeks of maintenance averaged close to that observed by Jackson ('15) in rats held at maintenance for seven weeks. This is slightly higher than the normal relative weight (10.4 per cent.) at three weeks, the beginning of the maintenance period. In the female rats refed one week the tract and contents formed 16.2 per cent. of the body weight, as compared with 15.4 per cent. noted by Jackson ('13) for normal rats of corresponding body weight (50.4 grams net). At the end of four weeks of refeeding, the relative weight of the stomach and intestines (13.5 per cent.) for the test rats was considerably above that (9.0 per cent.) in the controls. In the adult rats, the relative weight of the tract and contents for the controls and test rats respectively was 6.0 and 6.3 per cent. in the males, and 8.5 and 8.8 per cent. in the females.

In general therefore it may be concluded that the stomach and intestines including contents were heavier than normal for corresponding body weight at the end of the fasting period, but were practically normal at the end of two weeks of refeeding, and thereafter. Considerable allowance must be made for individual variations, which probably account for the apparent increase at the fourth week of refeeding.

19. *Suprarenal Glands* (*Table V.*).

The weight of the suprarenal glands in the male and female rats killed after maintenance for nine weeks exceeded Donaldson's norm plus 36.0 and 16.1 per cent., respectively. The relative weights of the glands (.054 per cent. for the female and .035 per cent. for the male) shows that sexual differentiation in weight had appeared. An increase with sexual differentiation in the weight of the suprarenal glands in young rats held at maintenance was likewise observed by Jackson ('15).

Following refeeding, the weight of the suprarenals was constantly below the Wistar norm to a variable extent for the first four weeks. In the controls at sixteen weeks of age, the suprarenals were slightly (4.2 per cent.) above the norm. This would indicate that the excess weight accumulated by the suprarenal glands while the body weight remained constant did not persist; but on the contrary the glands appear to lag behind their normal proportions in the growth of the body upon refeeding. It is of course barely possible that this deficiency in the weight of the suprarenals in the refed rats may be due to chance variations.

In the adult rats, the suprarenals were also below Donaldson's norm, but as compared with the controls the glands were slightly heavier in the test animals. The differences however are small, and probably not significant.

It may therefore be concluded that the suprarenals, in which growth is persistent during maintenance, lag behind upon refeeding and appear even to drop below the norm. In those refed to the adult stage, however, there is no marked difference between test animals and controls.

20. *Kidneys* (*Table V.*).

The kidneys appear to be slightly below normal weight for corresponding body length at the end of nine weeks of underfeeding. The differences, however, are small and probably not significant. Jackson ('15) observed that in rats held at constant body weight there was a slight tendency to increase in the weight of the kidneys, in the earlier weeks, but little or no apparent difference later.

During the first four weeks of refeeding, in the majority of cases the kidneys were slightly heavier than Donaldson's norm. At sixteen weeks, the kidneys were 23.5 per cent. above the norm in the female refed rats, and 15.4 per cent. in the controls.

In the adult rats the kidneys in both test animals and controls were close to Donaldson's norm. It is evident therefore that the kidneys were practically normal in weight, both at the end of the fasting period, and throughout the period of refeeding.

21. *Testes and Epididymi* (*Table V.*).

On account of the small number of observations, conclusions concerning the testes and epididymi are rather unsatisfactory during the early periods of refeeding. As compared with Donaldson's norm, the testes appear to be constantly below normal weight, especially at the end of the maintenance period (contrary to the observations of Jackson). In the adult rats, however, these glands are practically normal as compared with the controls.

The epididymi are not included with the testes in Table V. Their relative weight (.092 per cent. of net body weight) in the male rat killed at the end of nine weeks of maintenance, was close to the average given by Jackson ('15) for the normal at three weeks (.084 per cent.) and in rats held at maintenance for seven weeks (.080 per cent.). At the end of four weeks of refeeding the relative weight of the epididymi (.12 per cent.) the net body weight being 117.4 grams, appears to be unusually low. In the adult rats the relative weight of the epididymi averaged .27 per cent. in the controls and .21 per cent. in the test animals.

On account of the great normal variability in the weight of both testis and epididymis, and the small number of observations, no conclusions can be drawn with any degree of certainty. However, the evidence indicates that the testes and epididymi were somewhat below normal weight during the early refeeding periods, but practically normal in the adult animals.

22. *Ovaries* (*Table V.*)

The ovaries were practically normal as compared with Donaldson's norm in the female rat at the end of the underfeeding period. Jackson ('15) noted an apparent decrease in the weight of the ovary during maintenance.

On refeeding, the ovaries were below Donaldson's norm (minus 11.6 per cent.) at the end of the first half-week, but exceeded it (plus 43.2, 44.5 and 27.9 per cent.) at one, two, and four weeks of refeeding. After four weeks of refeeding (16 weeks of age) the ovaries were practically normal in the controls. In the adult rats the ovaries in both test animals and controls were somewhat below Donaldson's norm.

It therefore appears that the ovaries on refeeding became heavier than the norm during the earlier periods, reaching a maximum excess weight during the second week, and decreasing to the norm, or below, before the adult stage was reached. However, the large amount of variability in the normal weight of the ovary (due to changes during the sexual cycles) makes any definite conclusions from the available data very difficult or impossible.

23. *Hypophysis* (*Table V.*).

The weight of the hypophysis at the end of the fasting period was 14.8 per cent. below Donaldson's norm in the male and 9.5 per cent. above it in the female. Jackson ('15) noted a distinct tendency for the hypophysis to increase in weight in young rats held at maintenance.

On refeeding, the hypophysis shows no constant variation from Donaldson's norm. At the end of the fourth week in the test females it was 13.5 per cent. above the norm, whereas the controls were 2.6 per cent. above. The difference is probably due to normal variation and of no special significance.

In the adult rats the hypophysis weight in both controls and test animals shows considerable variation from the Wistar norm, being especially high in the females.

The evidence therefore indicates that the hypophysis was practically normal in weight at all times during the process of refeeding.

24. *Pineal Body* (*Table V.*).

On account of lack of data for comparison, no conclusions can be drawn concerning the pineal body in the rats killed at the end of the underfeeding period, and in those refed one half, one, and two weeks. The observations are recorded in Table V.

In the test rats refed four weeks the gland was apparently normal, the relative weight being .00123 and .00111 per cent.

of the body weight in the male and females respectively, as compared with .00116 per cent. in the controls of the same age.

In the adult rats, the relative weight of the pineal body was .00042 per cent. in the control males and .00050 per cent. in the test males. For the females, the relative weight was .00066 per cent. in the controls and .00067 per cent. in the test animals.

The differences in relative weight between males and females are probably due to the difference in body weight. There is no indication of a sexual difference in animals of the same body weight (such as has been found in the suprarenals, hypophysis and parathyroids), which is in accord with the conclusion of E. R. Hoskins (at present unpublished) based upon data collected in this laboratory.

It appears therefore that the pineal body was of normal weight in the rats refed four weeks and one year.

CONCLUSION.

The present experiments have shown that the marked tendency of the body as a whole in young rats to recover its weight after a period of maintenance is likewise characteristic of the various organs and parts. The abnormal proportions produced by underfeeding rapidly disappear upon generous refeeding, so that practically the normal relations are restored in most cases within four weeks. This readjustment evidently involves a modification of the growth curves of many of the various organs and parts of the body during the early refeeding periods. Thus those organs and parts which lost weight during maintenance grow more rapidly than the body as a whole, whereas those which gained weight during the maintenance period show a tendency to lag behind until the normal balance is restored.

SUMMARY.

The more important results of the present investigation may be summarized as follows:

The average daily gain in weight of the young rats refed after being held at constant body weight for short repeated periods was somewhat higher than that of the controls. This enabled the test rats to make up lost time and to overtake the controls, but not to exceed them in body weight.

The average daily loss in weight did not increase in the rats on successive periods of severe fasting, involving a loss of 25 per cent. of the initial weight.

The increment of body weight upon refeeding the test rats after each severe fast amounted to only about 16 per cent. of the ingested food (exclusive of water).

The amount of food required daily for maintenance decreased during the first fifty days of the experiment, but after that time apparently no further diminution occurred.

The growth in body weight of the rats refed after maintenance for various periods averaged for some time considerably higher than the normal for the (younger) controls of corresponding initial weight. Thus the stunted rats were able to overtake the full-fed controls in body weight before the end of the normal growth period. No effect of the stunting upon the ultimate body weight was noted.

As to the body proportions, the relative weights of the head, trunk and extremities remain practically normal during the various periods of refeeding.

Of the systems, the musculature and "remainder" continue practically normal for corresponding body weight during the various periods of refeeding. The integument rapidly increases, and the skeleton decreases, in relative weight, so that both reach approximately the normal proportions within the first two weeks of refeeding after maintenance from three to twelve weeks of age.

The viscera which are known to lose weight during maintenance—thymus, spleen, thyroid, lungs and ovaries—likewise apparently regain their normal relative weight within two weeks after refeeding. The thymus, and possibly the lungs, spleen, and ovaries, are apparently even *above* normal (over-compensatory growth) at four weeks of refeeding, but all are found practically normal in the rats refed to the adult stage.

The viscera whose weight remains nearly constant during maintenance—brain, heart, kidneys, liver, and epididymi—in general present approximately normal proportions during the process of refeeding; although the heart appears slightly above normal and the epididymi somewhat below.

The viscera whose weight increases during maintenance—

eyeballs, spinal cord, alimentary canal, hypophysis, testes and suprarenals—have in general decreased in (relative) weight so as to approach the normal within the first four weeks of refeeding. The testes and the suprarenals may even become subnormal in weight during the first four weeks of refeeding, but all are practically normal in the rats refed to the adult condition.

The pineal body was approximately normal in weight in the test rats refed four weeks, and in the adult test animals. There was no evidence of a sexual difference in the weight of the pineal body in rats of corresponding body weight.

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